SUPPLEMENT

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THE WASSERMANN REACTION.

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BEFORE any laboratory is competent to carry out complement fixation tests a thorough acquaintance with a reliable hæmolytic system is obviously essential to success. We propose to deal first with the hæmolytic system, then with various factors of importance in the ice-box method or what we propose to call "the cold method," and finally to consider the results by various methods and by the flocculation test as carried out by Dreyer and Ward.

The Haemolytic System.

It seemed to us desirable to prepare a suspension of washed red cells which was approximately constant and the usual methods are not accurate. The following method adds only about ten minutes to the usual preparation time.

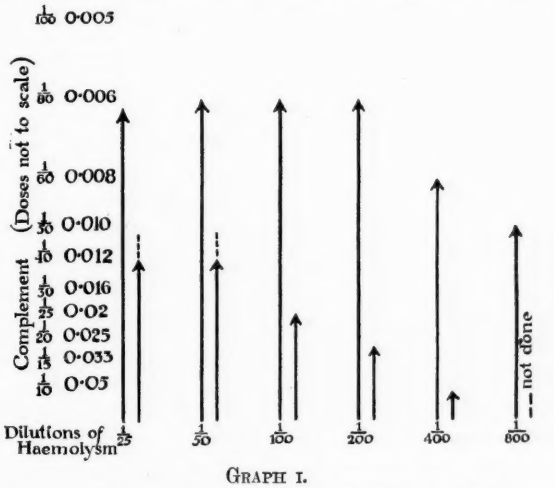
(i.) Make up an ample supply of one in twenty saline solution suspension of thrice washed red cells from defibrinated sheep's blood.

(ii.) Mix well; take 0.5 cubic centimetre into a graduated cylinder or test tube and make up to 12.5 cubic centimetres with saline solution.

(iii.) Mix well; pipette a drop on to a blood counting slide and count three or more sets of sixteen squares. If three sets are counted, the number of red cells in the standard suspension is two hundred and forty. If, for example, three hundred and twenty are found, the suspension is three hundred and twenty over two hundred and forty too strong and 33.3 cubic centimetre of saline solution will be added to each one hundred cubic centimetres of suspension. This standard selected by us contains approximately five hundred thousand cells per cubic centimetre.

We have found it difficult to keep red cells for the Wassermann test. The preserved cells supplied from the Commonwealth Serum Laboratories and said to be kept in Locke's solution, have been used by us in hæmolytic experiments after six months, but we are unable to state whether they are satisfactory as a substitute for fresh red cells in the Wassermann test. The hæmolysin (amboceptor) prepared by the above laboratories has been found satisfactory. Ampoules are only opened as required and batches with new label number are titrated.

In the original Wassermann method a small excess of hæmolysin was used (about two minimum hæmolytic doses), but English workers (Harrison, Griffith and Scott, McIntosh, Fildes) have favoured from five to twelve minimum hæmolytic doses. Graph I. shows the results



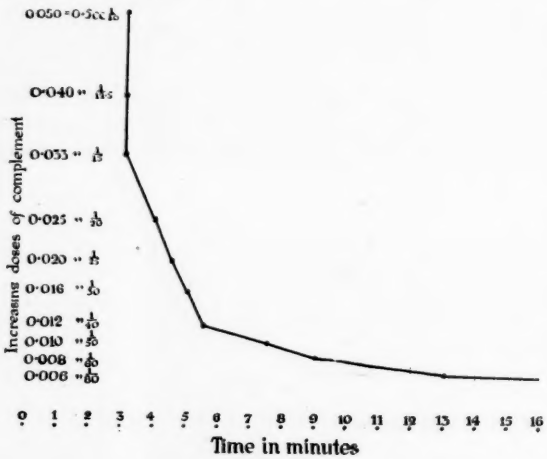
GRAPH I.

Titrations of Haemolytic Serum and Determination of the Excess Unabsorbed by the Corpuscles.

Standard emulsions of corpuscles were added to equal amounts of above dilutions of Haemolysin, and placed in ice box for 24 hours. Half of each batch of sensitized cells was then added in 1 ccm. amounts to diminishing doses of complement and extent of haemolysis is expressed by each taller line (on the left). The other half was allowed to settle for another 24 hours and was then centrifuged. The supernatant fluids were then added to same amounts of crude corpuscles and then to complement dilutions. The extent of haemolysis is shown by the right-hand lines (shorter).

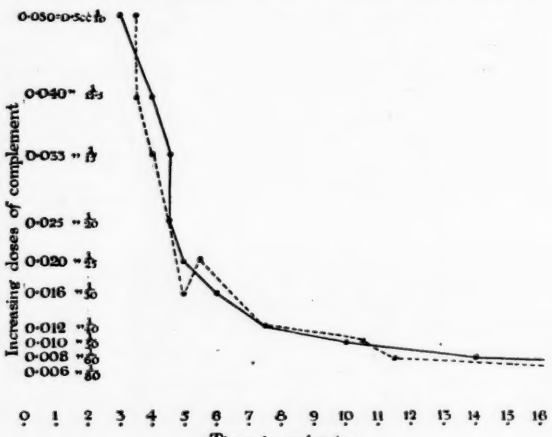
of a series of titrations of complement against standard red cells sensitized with falling amounts of hæmolysin. The batches of sensitized cells were placed in the ice-box (temperature 2° to 4° C.) for twenty-four hours, then well mixed and each divided into two portions, one of which was added to falling doses of complement and the end points determined (taller ordinates). The other portions were replaced in the ice-box till the next day, then centrifuged and the supernatant fluids added to identical amounts of crude washed corpuscles. Each batch was then added to falling doses of complement and the end points determined (shorter ordinates). It will be seen

that one in two hundred hæmolysin gives maximum sensitization and the excess lying free is definitely less than when one in one hundred is employed and a Neisser-Wechsberg type of deviation by excess of amboceptor is not likely to occur. According to this experiment, therefore, one in two hundred or somewhere between one in two hundred and one in four hundred is the optimum dilution of this hæmolysin, where maximum sensitization is desired (in our method equal parts of this dilution and standard suspension are mixed, so that the final concentration in the sensitized corpuscle suspension is one half the above). It is obvious that the labelled titre of proprietary hæmolysins means nothing unless the method of titration is known.



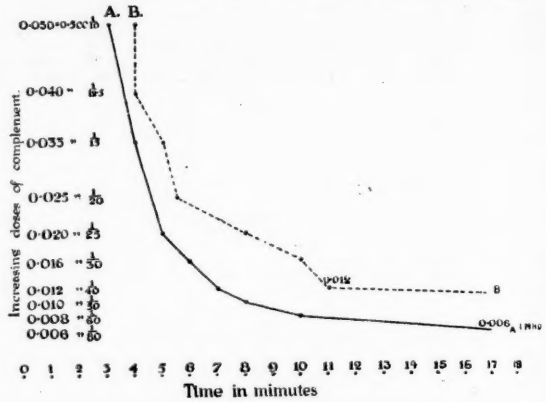
GRAPH II.
Titration of Fresh Complement in Water Bath at 37deg. C.

Complement varies in hæmolytic power in different guinea pigs and its titre seems to be depressed for periods as if there were some general influence on the stock. Kept



GRAPH III.
Comparison of Fresh Complement with the same after 30 hours at approx. 0deg. C.
Continuous line indicates fresh complement.
Interrupted line indicates same complement 30 hours later (kept about 0deg. C.).

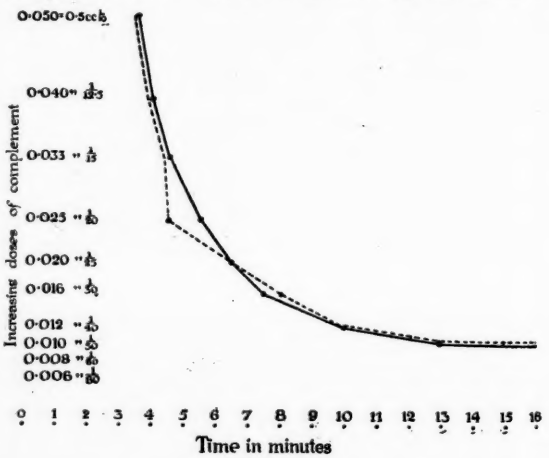
frozen in a "Thermos" flask, its titre does not drop much as a rule within forty-eight hours. Graph II. shows the end point and rate of hæmolysis of a complement against standard sensitized red cells. Hæmolysis was complete



GRAPH IV.
Comparison of Fresh Complement with the same after 24 hours at approx. 0deg. C.
A indicates fresh. 1 M.H.D. is .006 c.c.
B indicates same kept at 0 deg. C. approx. 24 hours later. 1 M.H.D. is .012 c.c.
Therefore 4 M.H.D. is .025 c.c., now 2 M.H.D. A depreciation of 50 per cent., or 2 M.H.D.

with the larger doses in three minutes and practically ceased in tubes with smaller doses in thirteen minutes. Some complements appear to be slower in action and the end point is not found till later.

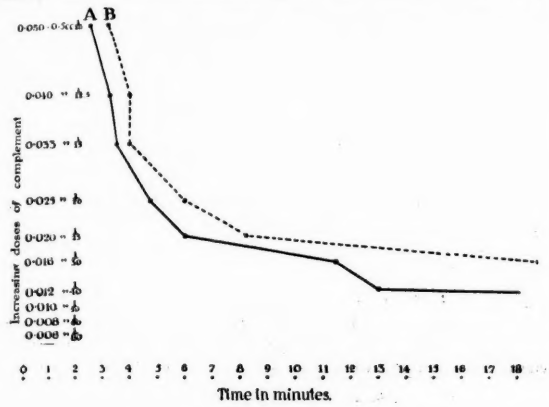
Graph III. shows that the complement kept frozen did not appreciably fall in titre in thirty hours, whilst in Graph IV. there is shown a distinct fall in rate of hæmolysis and in end point of another complement after twenty-four hours. These variations are, of course, of



GRAPH V.
Absence of anti-complementary effect of dilute cholesterinized antigen when the exposure is very short
Titration of Complement.
Two sets of dilutions of one complement were put up, saline added to one set (continuous line), Bordet's Extract and cholesterin added to other (interrupted line) and then sensitized corpuscles immediately added.
The strength of the extract was 1.0 cc. plus .1 cc. of cholesterin in 60 cc. saline.

importance in the cold method in which the complement must be kept for twelve to eighteen hours before its hæmolytic action is to be exerted.

In Graph V. is shown a comparison between a simple complement titration and an identical set to which were added single doses of antigen (Bordet and Ruelen's extract, one cubic centimetre, *plus* 0.1 cubic centimetre,

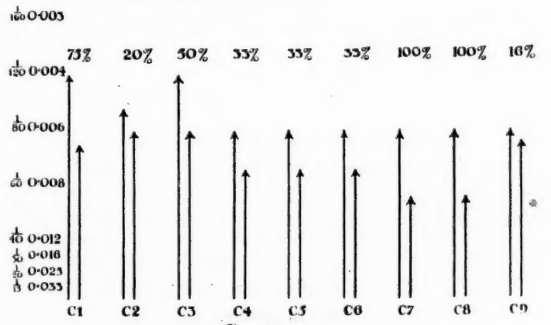


GRAPH VI.

Effect of Dilute Cholesterinized Antigen on Complement.
1 hour's exposure at 2-4deg. C.

Two sets of dilutions of one complement were put up, saline added to one set (continuous line), Bordet's Extract and cholesterin added to other (interrupted line), both placed in ice box for one hour and then sensitized corpuscles added.
A. 1 M.H.D. is .012 ccm.
A. 4 M.H.D. is .05.
B. 1 M.H.D. is .016.
B. 3 M.H.D. is .05.
Therefore a reduction of 4 M.H.D. to 3 M.H.D. (or one-third more complement) is necessary.

cholesterin in sixty cubic centimetres of saline solution). No appreciable effect was produced where, as here, no appreciable time elapsed before the sensitized corpuscles were added. The anti-complementary action of dilute cholesterinized antigens takes an appreciable time, although we found a definite depreciation in half an hour at 2° to 4° C. in one experiment and in one hour in another experiment (see Graph VI.).



GRAPH VII.

Variation in Sensitiveness of Nine Different Complements to a Dilute Cholesterinized Antigen.

These figures above ordinates show percentage increase in complement required to effect haemolysis when comp. has been exposed to antigen in ice box for 10-17 hours.
Titration of 9 complements to show varying sensitiveness to Bordet's extract and cholesterin. The taller lines on left give the end pts without antigen, the shorter ordinates give the end pts when antigen is present. The tubes were placed in ice box for about 11 hours in the case of five complements, for about 17 hours in the case of the remainder, and sensitized corpuscles then added.

Graph VII. shows the effect of dilute cholesterinized antigen on nine different complements, the length of exposure being, in the case of five for eleven hours and in the case of four for seventeen hours and the temperature 2° to 4° C.. The taller ordinates show the end points of the fresh complement and the shorter ordinates the end points after exposure. The figures above express in percentages the additional amounts of the same complement required to effect the same amount of lysis as did the fresh complement. The variations were from 16% to 100%.

In another series of experiments the effect on complement of varying the relative amounts of extract and cholesterin was studied, but no very striking differences in end point were noted between eight parts to one, four parts to one, two to one and equal parts, provided that the absolute amount of cholesterin in the antigen was not increased. If the amount of cholesterin is increased, whilst the amount of extract is kept constant, the depreciation of complement increases, though the presence of normal inactivated human serum tends to protect it and this protection is of great importance in the Wassermann test. In one careful experiment, it was found that of four minimum hæmolytic doses of complement exposed to Bordet and Ruelen's extract four parts, cholesterin one part (the dilution of the latter being one in three hundred), for a period of twelve hours at 2° to 4° C., no less than 2.3 minimum hæmolytic doses had disappeared, only 1.6 remaining.

In the actual Wassermann tests, however, the protective effect of the sera to be tested comes into play and we are able to use smaller doses of complement. If one is working with a sensitive system in the sense of having only a little excess complement, one will often find that the antigen controls (containing no serum) will not show hæmolysis, although all the sera may completely fail to yield reaction. And further we are of the opinion that "very weakly positive" sera will not always produce as much fixation by such sensitive methods as does the antigen alone and one wonders whether such sera should be described as "very weakly positive" sera or sera lacking in protective effect on complement. These sera very commonly come from treated patients and the evidence is in favour of the specific nature of this very weak reaction if the serum itself is not anti-complementary.

We have tried many variations in the proportion of extract to cholesterin and were not able to determine any optimum relation for all sera. At present we are using a proportion of four parts of extract to one of cholesterin, as contrasted with Griffith and Scott who use equal parts, but we do not regard this question as closed. For quantitative results we have tried using falling doses of antigen in the test; we have also tried falling doses of test serum, but we are still using varying doses of complement and keep other reagents constant. This question is also open for discussion.

Comparison of Warm and Cold Methods with 1227 Sera.

These sera did not by any means all come from patients with syphilis; many were examined more or less as a routine hospital measure to exclude syphilis. Many tests were done as a check on treatment. In 90% of sera ap-

proximately identical results were obtained by both methods, that is to say, 69% were frankly "negative" and 21% fixed the maximum dose of complement used (five or six doses only). Considerable difference between end-points by the two methods might have been disclosed if this had been attempted, but we have not made this a routine, because by our method it would have necessitated the use of very large numbers of guinea pigs. In

TABLE I.

Method		Number	Per centage	
Cold	Warm			
—	—	845	68.9	90
+ + +	+ + +	259	21.1	
+ + +	+ + or +	60	4.9	10
+ + +	—	37	3	
+ + or +	—	21	1.7	
+ +, + or —	+ + +, + + or +	5	.4	

10% of sera divergent readings were obtained and 9.6% of these gave more fixation by the cold method than by the warm. We believe that all of these are really "partial positives" and that the greater length of time during which the serum and antigen interact in the cold method (twelve to eighteen hours) allows more fixation of complement than in the warm method (one hour). Very occasionally a "weakly positive" serum will give more fixation in the warm than in the cold (the same complement being used) and we endeavoured along these lines to find some qualitative difference between "Wassermann substance" in different sera, but we came to no definite conclusions.

In another series of one hundred sera that were "positive" in some degree by the cold method, 68% were "strongly positive" by the warm method, 16% were "weakly positive" by the warm, though "strongly positive" by the cold, 9.7% were "negative" by the warm, though "strongly positive" by the cold, and 5% were "negative" by the warm, though "weakly positive" by the cold.

Of forty-eight sera in one series which gave a reaction by the cold method and failed in the warm, nineteen were from treated patients, six were primary syphilis, six were tabes and the remaining seventeen were from patients presenting very varying clinical pictures.

Criticisms of Recent Comparisons of the Wassermann Test With the Flocculation Test.

In a recent publication by the Medical Research Council (1), Dreyer, Ward, McIntosh and Fildes conclude:

(1) That an approximately equal number of positive results is obtained by the Wassermann and "Sigma" reactions in untreated cases of manifest syphilis.

(2) That in treated cases the "Sigma" reaction gives a larger number of positive reactions than the Wassermann reaction. This is particularly obvious in cases giving readings of small "Sigma" unitage.

Their tables (page 8) show that of four hundred and forty-six "cases" of syphilis, treated and untreated, and including forty-one cases labelled "serological syphilis," 61% gave a Wassermann reaction and 73% a "Sigma" reaction (positive flocculation). The latter method picked up reactions, therefore, in 12% of "cases" in which the Wassermann test failed. This comparison is of slight importance because the Wassermann tests were carried out by McIntosh and Fildes separately, but both by the warm method and the latter fails in comparison with the cold method for the same reason that the warm method fails in comparison with the flocculation test. The reason is to be found in the time of interaction of serum and antigen. McIntosh and Fildes gave one hour at 37° C., whilst Dreyer and Ward took readings after twenty to twenty-two hours and after forty to forty-four hours in a water-bath at 37° C.. This is obviously quite unfair to the Wassermann test and it is somewhat surprising that such a comparison should have been made. We do not in any way wish to question the value of the flocculation test nor the useful and careful work of Dreyer and Ward in this connexion.

Reference.

(1) Medical Research Council, Special Report Series, No. 78, 1923. "The Serum Diagnosis of Syphilis; the Wassermann and Sigma Reactions Compared."

THE EFFECT OF VARIATIONS IN FIXABILITY OF COMPLEMENT ON THE WASSERMANN REACTION.

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I PROPOSE discussing the relation which variation in the behaviour of complements bears to the result in the Wassermann test. It has been recognized for some time that the two functions of complement in the reaction, that is hæmolytic activity and fixability, are independent of each other.

For this reason complement is of all the reagents used in the test the most difficult to standardize and therefore variations in these activities as manifested by different complements are most likely to prove an important factor in the production of discrepant or varying results.

Standardization of complement in regard to hæmolytic activity has for long been practised as a preliminary to determining the dose to be used in the test proper—sensitiveness to antigen alone being also estimated at the same time.

Recent work has, however, shown that, provided selected guinea pigs are used for the supply of complement, variations in hæmolytic activity fall within well defined and narrow limits and that such variations have little effect on the result of the Wassermann reaction and can be disregarded. Further, it has been shown that sensitiveness of complement to antigen alone and specific fixability in the test proper do not run parallel, for example, a complement which is abnormally sensitive to antigen as determined in the preliminary test, may be less fixable, that is give a weaker Wassermann reaction when compared with an insensitive complement used in the same amount. A further difficulty has been added by the introduction of the ice-box method of fixation in which, owing to the prolonged period of fixation, preliminary standardization of

complement as regards hæmolytic activity or sensitiveness to antigen is precluded. For these reasons "the method of adjusting the amount of complement so that it may represent an arbitrary standard of hæmolytic activity both alone and in the presence of antigen cannot be relied upon to produce uniformity of results in the Wassermann reaction."

Griffith and Scott, who recently carried out a most comprehensive investigation into the rôle of the various factors concerned in the reaction, advocate the use of a fixed dose of complement, the latter being obtained from healthy male guinea pigs only. As they point out, the use of a quantity considerably in excess of the minimum complying with the necessary requirements involves a diminution in the delicacy of the reaction with consequent failure to detect certain cases of syphilitic infection. "Such an excess also tends to exaggerate the inevitable variations in the result which depend upon inherent differences in the fixability of complements." The standard dose recommended is 0.01 cubic centimetre used as one volume (0.25 cubic centimetre) of a one in twenty-five dilution, no attention being paid within certain limits to the hæmolytic activity or to sensitiveness to antigen alone.

In actual practice I have found this amount to represent approximately two minimum hæmolytic doses of complement measured in terms of hæmolytic activity, provided that healthy male pigs only are used. (I take the further precaution of starving the animals for twelve or eighteen hours before killing them).

The irregular sensitiveness to antigen alone, though as Griffith and Scott point out it is only exceptionally a special disturbing factor, is obviated by using an antigen which rarely shows anti-complementary properties.

An acetone—soluble—free extract prepared either according to the method of Noguchi or Bordet and Ruelen is used, to which cholesterin in known amount is added immediately before use.

The avoidance of an excess dose of complement has undoubtedly increased the delicacy of the reaction, but even if the standard dose be used as suggested, variations in result may be obtained with the one serum when tested with several different complements in the presence of the same antigen suspension. These variations, regarded by Griffith and Scott as inevitable, are due to differences in the ease with which the various complements are fixed in the specific reaction. So long as variations in fixability are not extreme, the test results will not differ to any extent, but occasionally complements are met with of such low powers of fixability that, unless the technique can be adjusted to compensate for such a possible contingency, discrepant and incorrect results will obtain.

The following experiments will illustrate the different degrees of fixability shown by different complements and will help to make clear the reasons for a suggestion to be made below, for adjusting the technique so that some indication may be obtained of the degree of fixability of complement in carrying out the reaction.

If one titrates an antigen separately with each of several complements in the presence of the same positively reacting serum, it is noted that the minimal dose of antigen that will effect complete inactivation varies with the different complements.

This minimal dose of antigen is spoken of as the antigenic unit.

In Experiment I. a syphilitic serum was tested in this way with four different complements, a Noguchi extract with cholesterin added being used as antigen. The same amount of patient's serum was used in each tube and the dose of complement was 0.01 cubic centimetre throughout.

The results shown in Table I. with the various dilutions of antigen were obtained.

TABLE I.

Comparison of four complements in respect of capacity to be inhibited by antigen + a syphilitic serum.

Complement		Syphilitic Serum	One Volume Antigen (Noguchi extract + 1% cholesterin in equal parts) diluted as under								
Designation	1 Volume Diluted		1 in 2240	1 in 2560	1 in 2880	1 in 3200	1 in 3520	1 in 3840	1 in 4160	1 in 4480	1 in 4800
A	1 : 25	1 : 5	0	0	0	0	0	0	Tr.	Tr.	1+
B	1 : 25	1 : 5	0	0	Tr.	1+	1+	2+	3+	3+	3+
C	1 · 25	1 : 5	0	0	0	Tr.	Tr.	1+	1+	1+	2+
D	1 : 25	1 : 5	0	0	0	Tr.	Tr.	Tr.	Tr.	1+	2+

One volume of syphilitic serum (one in five) and one volume of complement (one in twenty-five) were used in each test. Fixation in the ice-chest was for eighteen hours—then one volume of the sensitized cells (maximal sensitization) was added.

0 = No hæmolysis, supernatant fluid quite clear when corpuscles have settled.
Tr. = Trace of hæmoglobin in the supernatant fluid after the corspuscles have settled.
1+ = One-fifth of the cells lysed.
2+ = Two-fifths of the cells lysed. *et cetera.*
C = Complete lysis.

The complements titrated alone for hæmolytic activity gave the minimum hæmolytic dose as follows:

Complement A. 0.005 c.cm. (= 0.25 c.cm. of 1 in 50)
Complement B. 0.004 c.cm. (= 0.25 c.cm. of 1 in 60)
Complement C. 0.005 c.cm. (= 0.25 c.cm. of 1 in 50)
Complement D. 0.004 (= 0.25 c.cm. of 1 in 60)

None of the complements were sensitive to antigen in a concentration of the latter as high as 1 in 640, with the minimal hæmolytic dose of each complement.

The experiment shows that complement A. is more readily fixed than any of the others, so that complete inactivation occurs with a dilution of antigen up to 1 in 3,840. Complement B exhibits the lowest degree of fixability, inactivation occurring only up to a dilution of 1 in 2,560. It is obvious that this divergence in results is not due to differences in hæmolytic activity for complements A and C have the same hæmolytic value, the dose used of these two representing two minimum hæmolytic doses in each case. Yet we find the antigenic unit with the one is 1 in 3,840 and with the other 1 in 2,880.

The same difference is noted with complements B. and D which are equal in hæmolytic value and it is seen that hæmolysis proceeds with much greater rapidity with complement B in the higher dilutions of antigen than with complement D, that is in all dilutions of antigen complement D exhibits a greater facility for becoming fixed in the specific reaction.

The next experiment illustrates the same difference in fixability when crude antigen is used. The antigen of McIntosh and Fildes was used with 1% cholesterin added in the proportion of three parts of extract to two parts of cholesterin. In this experiment the dose of complement used was adjusted to the hæmolytic activity, two minimum hæmolytic doses being used in each case (see Table II.).

This experiment is also of interest as it shows that the complement of higher lytic activity is more readily fixed and therefore produces less hæmolysis than the complement of lower lytic activity and illustrates well the fallacy of attempting to compensate in the test for differences in hæmolytic activity by using less of the more active complement without having some knowledge of its fixability.

Experiment No. III. shows similar variations in fixability when complement is used in excess with a crude antigen (see Table III.).

In this experiment complements A and C were of equal lytic power, but differed from complements B and D which were also equal in lytic activity. Yet complement C is fixed more readily than complement A and complement D more readily than complement B . Complements B and C though differing in lytic activity, were each fixed to the same degree in the specific reaction and this whether three or four minimum hæmolytic doses were used.

If a cholesterin free antigen is used, such as the Bordet and Ruelen antigen, the same variation in fixability is noted.

It is obvious from these experiments that the antigenic value of any antigen varies with the fixability of the complement with which it is used, the antigenic unit in each case being dependent on this activity of complement. This

TABLE II.

Comparison of two Complements in Respect of Capacity to be Inhibited by Crude Antigen plus a Syphilitic Serum.

Complement			Syphilitic Serum	Antigen					
Designation	M.H.D.	Volume c.cm.		1;50	1;60	1;70	1:80	1;90	1:100
A	2	0.008	1;5	0	0	0	0	Tr.	1
B	2	0.010	1;5	0	Tr.	Tr.	1+	2+	4

The antigen was McIntosh and Fildes's with 1 per cent. cholesterin (3:2). One volume of sensitized cells was added after eighteen hours' fixation in the ice chest (maximum sensitization).

TABLE III.

Comparison of four Complements in Respect of Capacity to be Inhibited by Crude Antigen plus a Syphilitic Serum when excess of Complement is used.

Complement			Syphilitic Serum	Antigen				
Designation	M.H.D.	Volume c.cm.		1:30	1:40	1:50	1:60	1;70
A	3	0.018	1:5	0	0	Tr.	Tr.	1+
B	3	0.015	1:5	0	0	0	Tr.	Tr.
C	3	0.018	1:5	0	0	0	Tr.	Tr.
D	3	0.015	1:5	0	0	0	0	Tr.
A	4	0.024	1.5	0	Tr.	Tr.	3+	4+
B	4	0.020	1:5	0	0	Tr.	1+	3+
C	4	0.024	1:5	0	0	Tr.	3+	4+
D	4	0.020	1:5	0	0	0	Tr.	Tr.

The antigen was McIntosh and Fildes's with 1 per cent. cholesterin (3:2). One volume of sensitized cells was added to all tubes after eighteen hours' fixation in the ice chest (maximum sensitization).

TABLE IV.

Comparison of Two Complements in Respect of Capacity to be Inhibited by Cholesterin-free Antigen and Syphilitic Serum.

Complement			Syphilitic Serum	Antigen			
Designation	M.H.D.	Volume c.cm.		1:640	1.800	1;960	1.1120
A	2	0.01	1;5	0	0	Tr.	Tr.
B	2	0.1	1;5	0	0	0	Tr.

The antigen was Bordet and Ruelen's without cholesterin. One volume of sensitized cells was added to each tube after eighteen hours' fixation in the ice chest (maximum sensitization).

offers a means for standardizing antigen in the direction of determining its unit of antigenic value for what might be regarded as normal complements, *id est* complements with a normal range of fixability, and for some time now I have been using this method in standardizing the antigen I use.

Experiment No. III. shows that the four complements used, though differing in power of fixability, showed no obvious variation. These may be regarded as normal complements.

Experiment No. I. shows one complement (complement A.) with a high degree of fixability, but in the other three, variation is only slight, and since abnormal complements are rarely encountered, it may be taken that the three complements B, C. and D. are of normal range.

The antigenic unit for any particular antigen is determined by titrating out the antigen with a fixed dose of four or more complements in the presence of one (or more) strongly reacting serum. For this purpose I used pooled serum from several untreated persons suffering from active syphilis. The dose of complement used in this titration must be the same as that used in the reaction and the method is impracticable if the dose of complement is varied on each occasion to represent an arbitrary standard of hæmolytic activity. Using several complements the average antigenic unit of the antigen can be determined with complements of normal range of fixability.

With the Noguchi extract which I use with 1% cholesterin added in equal amount, the antigenic unit has been remarkably constant with several different antigens and ranges betweena 1 in 4,800 concentration and a 1 in 5,120 or an average of 1 in 5,000. In the test proper ten times the antigenic unit is employed, this dose being based on the results of Kolmer's work in this respect. The dose of this antigen used in the test proper is, therefore, one volume (0.25 cubic centimetre) of a 1 in 500 suspension. With such a dose one would expect that with strongly reacting sera complete inactivation will occur with all complements whose range of fixability lies within a concentration of antigen of 1 in 500 to 1 in 5,000.

With weakly reacting sera, however, a complement whose antigenic unit with a strongly positive serum approximated the 1 in 500 concentration (that is a complement of extremely low power of fixability), may be expected to fail to show any binding in the reaction. With a view to ascertaining if this were so, I have for some little time included an extra row of tubes in the test in which the dose of antigen is twenty-five times the antigenic unit (= 1 in 200 with the antigen described above).

Though I have only had the opportunity of testing a few sera in this way, the results appear to justify the procedure.

In a series of one hundred tests carried out during the past four months, five complements have been met with out of a total of twenty-four used which appear to have been abnormally low and with which evidence of any reaction to the test was only obtained (in the case of weakly reacting sera) in the extra row containing the dose of antigen equal to twenty-five antigenic units. If the tubes containing a dose of antigen equal to ten units show complete lysis and the extra tube mentioned shows hampering, then the serum should be re-tested with another complement, the inference being that the first complement used was of low fixability. This will be confirmed at times by a weaker reaction occurring in the positive control tube, as has happened twice in my series.

On repeating these sera on each occasion with another complement definite evidence of reaction was obtained in the tubes containing the weaker suspension of antigen.

The results are shown in Table V.

TABLE V.

First Occasion of Testing—Re-testing with Different Complement.

Serum	Row				Row			
	I	2	3	4	I	2	3	4
No. 34	C	C	C	2+	Unable to repeat			
D.G.	C	C	C	I+	C	0	Tr.	0
No. 43	C	C	C	2+	C	0	I+	0
A.J.	C	3+	4+	0	C	0	0	0
No. 79	C	2+	4+	0	C	0	0	0

Row 1 = Serum control tube (complement 0.005 c.cm.)
Row 2 = Serum 1 in 5; antigen ten units (complement 0.01 c.cm.)
Row 3 = Serum 1 in 10; antigen ten units (complement 0.01 c.cm.)
Row 4 = Serum 1 in 5; antigen twenty-five units complement 0.01 c.cm.)

In each of the above cases the clinical history was strongly in favour of a syphilitic infection being present, yet on the first occasion of testing it would have been regarded that the serum had not reacted or in two cases that the reaction was doubtful, but for the hampering which occurred with the stronger concentration of antigen.

As a result of the experiments carried out by me I have come to the conclusion that discrepant results may occur even when a highly sensitive technique such as that of Griffith and Scott is used, this discrepancy being due to differences in fixability of complement. A possible means of obviating this is to standardize the antigen on the lines suggested and in the test proper to include an extra row of tubes—one for each serum—in which a dose of antigen is used equal to twenty-five times the unit.

Any sera which show hampering in this tube should be retested with another complement.

The control tests to insure that the lytic activity of complement and sensitiveness to antigen alone are not abnormal, are put up as suggested by Griffith and Scott, an extra control row being included for the larger dose of antigen.

Dr. C. Badham referred at some length to the methods employed at his laboratory. He favoured the method of Griffiths and Scott. He made a plea for the adoption of a standard method in conjunction with any other method which might be used in laboratories. Such a method could with many advantages be that of Griffiths and Scott. If the divergence of results of the test from different laboratories continued the confidence of practitioners would be shaken. The speaker did not wish to see any one test employed to the exclusion of all others, but only one standard test as he had indicated as a final court of appeal. He described his technique in performing the test.

Dr. R. J. Bull expressed his appreciation of the very considerable amount of valuable research work undertaken by the previous speakers in the routine carrying out of the Wassermann test. The same spirit of research characterized the performance of the test in the Melbourne University Bacteriological Department and other laboratories in Melbourne and elsewhere. All this must inevitably lead to a better appreciation of technical difficulties and their correction and to greater accuracy in interpretation of the results.

Personally he had experienced no difficulty in reference to red cells, but he agreed with Dr. Shearman in regarding complement as the most difficult factor in the test. Male guinea pigs were also the more satisfactory to work with. He never pooled guinea pig sera, but tested each individually. Complement varied considerably not only in hæmolytic activity, but in sensitiveness to antigen. Varying degrees of Wassermann reaction might be obtained with one serum by using different complements. Dr. Bull's practice was to obtain sheep red cells and complement on the day of performing the Wassermann test; he also prepared his own hæmolytic serum and antigen. When immunizing sheep antibodies other than hæmolysins were produced, such as agglutinins. On one occasion the latter were so evident that the red blood cells agglutinated and were resistant to hæmolysis in the Wassermann test. He found that by saturating the hæmolytic serum with red cells and using the supernatant fluid, satisfactory results were obtained with this treated hæmolytic serum in the Wassermann test. His results with warm and ice box methods accorded fairly well with those of Dr. Tebbutt. But in the few instances in which the two methods differed, who was to decide which results should be accepted? Personally he favoured a very full cooperation between clinician and pathologist, the verdict of the clinician to be the determining factor in interpretation of results.

As to Dr. Badham's suggestion of the use of one standar dmethod as a referee in cases where discordant results occurred by other methods, there was still the same difficulty as to the precise significance of the results. He was of the opinion that results sometimes differing in kind or in degree obtained with the same blood by different workers, did good by leading to discussion and further investigation. But on the whole it seemed a better practice to gauge the effects of anti-syphilitic treatment by comparing the results obtained from time to time by one worker rather than by several. One difficulty experienced with the ice box method was that occasionally in the controls the complement was entirely fixed by the antigen. Granted that this non-specific fixation differed from specific fixation by syphilitic serum and that the addition of human serum in the test seemed to correct this fixation, difficulty would arise in accepting the findings. When this occurred, the results of cerebro-spinal fluid tests could not be accepted as they are all positive, whether from specific or non-specific cases. His practice was to perform the test by the warm method and to repeat all which gave anomalous or doubtful results by the ice box technique, especially those cases exhibiting signs of involvement of the central nervous system.

Over 100,000 Wassermann tests had been made at the University Bacteriological Laboratory, an average latterly of 15,000 to 20,000 tests *per annum*. It was obvious, therefore, that with a small and overworked staff and insufficient financial assistance the test must be simplified as much as possible. He was pleased to note Dr. Badham's plea for use of one fixed volume of complement, instead of a multiplicity of tubes. A considerable degree of sensitiveness and specificity was obtained by the speaker by rather supersensitizing the red cells and using a somewhat small amount of complement. However, he believed that in the not distant future the Wassermann test would be replaced by a flocculation or similar test, in which equally valuable results would be obtained and simplified from the point of view of using fewer interacting colloids.

PRESIDENTIAL ADDRESS.

CANCER—REDUCTION IN ITS MORTALITY, ITS ETIOLOGY, THE CAPTIVE TISSUES, SUPERMALIGNANCY AND RODENT ULCER.

By J. Burton Cleland, M.D., Professor of Pathology, University of Adelaide,

Reduction in the Mortality from Cancer.

The principal object aimed at by all those engaged in the study of malignant growths is a reduction in the mortality from these tumours. It is possible that with further knowledge such reduction of the mortality may be achieved in a number of different ways, the summation of whose effects may lead to the saving of many lives and enable cancerous processes to be relatively controlled. It is, however, doubtful whether any means will ever be discovered for completely controlling or preventing these conditions.

As regards the ætiology of cancerous and other allied conditions, investigation may eventually show that a controllable factor exists and by the removal of this factor examples of these neoplasms will not appear, or if they do, they may be easily destroyed. For instance, it is possible that the cancerous process is really due to invasion of certain cells by very minute parasites and that the bizarre cell reactions that follow, are entirely due to the stimulation exercised by such parasites. It may be possible to devise means to destroy this virus or to render individuals immune to it and thus malignant processes would, for all practical purposes, cease to cause anxiety. Few workers on the subject, however, now think it likely that such cancer-causing, not merely cancer-predisposing, parasites exist. If the theory elaborated later in this paper is the true explanation of cancerous processes, then it would seem practically hopeless to be able to control the disease through our knowledge of its ætiology.

In surgery more perfect technique, the devising of the

most suitable operations for particular cases and readier means for the earlier diagnosis of malignant growths of the internal organs, may all lead to a material reduction in the cancer death rate.

In therapeutics it is possible that some selective drug or other substance may be discovered which is capable of destroying the malignant cells, leaving the neighbouring normal cells relatively uninjured. The value of X-rays and radium in the treatment of malignant growths has already led to most important results and the relatively easy control of certain types of such growths.

A fuller knowledge of the predisposing causes of cancerous growths may enable us to reduce the incidence of cases to a very great extent. There are many types of cancer that we know develope as a result of various forms of chronic irritation. Squamous epitheliomata of the tongue and of the lip, soot, tar and paraffin cancers, squamous epitheliomata developing from exposure to the X-rays, epitheliomata developing at the sites of old burns and many others in man are well known; whilst in rats, we have the developement of squamous epithelioma in the stomach as the result of the irritation of the worm *Gongylonema neoplasticum.*

We also know now that we can produce certain malignant growths at will by applying certain forms of chronic irritation to animals. Thus, the worm just mentioned in the rat can be administered in its larval stage in cockroaches with the certainty that in some cases a malignant growth will develope; mice may be painted with tar and epitheliomata thus produced and so on.

The frequency with which certain epithelial malignant growths can be traced to particular antecedent forms of chronic irritation, suggests the probability that all or very nearly all other forms of cancer owe their developement to some kind of chronic irritation which has not yet been recognized. In other words, were we only aware of all the various forms of chronic irritation that predispose to and lead up to the developement of malignant growths in various parts of the body, we should probably be able to remove some of these and to neutralize the ill effects of others and in that way stem materially the tide of cancer cases. It is almost certain that cancer of the stomach, cancer of the breast and cancer of the uterus all arise from different forms of chronic irritation. The nature of this chronic irritation may be physical or chemical or both. A thorough study of the conditions under which cases arise, may enable us to detect the active component that does the damage. I feel certain that it is well worth while to pay the utmost attention to the question of these predisposing causes and I feel that a reduction in cancer mortality is more likely to be achieved by work along these lines than along other avenues. In Australia it should be possible to make a collective effort to tackle this aspect in some forms of cancer.

Another means of reducing mortality from cancer is by education of the people in such a way that malignant growths may be brought under the notice of the surgeon at the earliest possible moment. It is a very difficult matter indeed to know what information should be communicated to the laity and how such information should be brought under their notice. There is little doubt, however, that much can be done in this connexion without giving rise to undue and morbid introspection. Undoubtedly when the generality of individuals are made aware of the nature of the symptoms and signs that raise any suspicion of the presence of a cancerous growth, medical attention will be sought in many cases earlier than at present with much better prognosis resulting. Associations are being formed in Britain and America with the object of dealing with this aspect of the cancer question. It might be considered with advantage also in Australia.

The Aetiology of Cancer; a Gametoid Theory.

In studying the behaviour of malignant growths the most striking histological feature is the infiltration of the surrounding tissues by the cells of the neoplasm. These cells, though departing more or less widely from the normal size, shape, general appearance and regularity of the mother cells from which they have been derived, nevertheless retain in nearly all cases some features by which their origin can be surmised. From this we may infer that, whether or not the cells have reverted to a more embryonic type, they still retain some at least of the differentiating somatic factors that have become associated with them during their ontogenetic developement. But though still retaining some special, we might say specific, differentiation, the malignant cells have departed widely from the parent cells in that they have broken through the normal barriers that confined them to certain situations and are now invading tissues into which the healthy cells are in some way restrained from entering. In this respect the malignant cells seem to have acquired—or rather let us say to manifest—a feature which the normal cells, even if possessing it, do not exhibit. The new tissue —the cancerous or sarcomatous tissue—no longer seems controlled, as regards the number of the cells or their relationship with neighbouring cells, in the same way as are the normal tissues, in which we find an orderly arrangement and a definite mass wonderfully correlated with the requirements of the individual.

Let us consider for a moment this orderly arrangement and this mass effect. During the developement of an individual the tissues and organs grow in size *pari passu* with each other. They are undoubtedly coordinated with each other. There are, of course, slight variations about the mean, but we do not find one organ, say the liver, growing on to an indefinite size and becoming out of proportion to the kidneys or the limbs. Very obviously some check is interposed upon the continued proliferation of the cells of the organs and tissues after they have reached a certain size. For instance, a lizard's tail grows to a certain length dependant on the species. If this tail be removed, the disappearance of the tail tissues upsets the equilibrium and from the stump a new tail grows which will reach, more or less, the original size and will then cease to grow further. In human pathology we meet with some very striking instances of this "'making good" of lost tissues, in the shape of some examples of compensatory hypertrophy. In the Pathological Museum at the University of Adelaide are two examples of hydatid infestation of the liver. In one case the entire left lobe, in the other case practically the entire right lobe, has been destroyed by the growth of the hydatid cysts. To compensate for the considerable destruction of liver substance we find that in each of these cases the un-

affected lobes of the liver have undergone a considerable hypertrophy, presenting very striking pathological specimens. This hypertrophy would seem to depend upon the destruction or removal of a large mass of liver substance. The result has been that the mass effect of liver substance present becoming less than normal, the remaining healthy cells underwent increase in number, until approximately the original bulk had been restored, when the restraining influence, whatever it was, brought again the process of multiplication to an end, just as originally the liver when it had reached the adult size, was restrained from growing any further. In the case of the destruction of part of the liver by hydatid cysts, we find that again, just as in normal developement, the bulk of tissue that is newly formed to compensate for the loss, bears a definite proportion to the other cells and tissues of the body. We do not find that the cells which undergo proliferation to make up for the lost tissues go on dividing indefinitely. A check to their further multiplication comes when approximately the right mass of tissue has been formed. Similarly in the kidneys we may find that atrophic changes and complete destruction of kidney parenchyma have occurred on one side and that the kidney on the other side has undergone a compensatory hypertrophy and has much increased in size in consequence.

In studying the hydatid livers just mentioned we also see that the reason why one or other of the lobes of the liver reaches normally a certain size and stays there, is not due to any physical or nutritive factor preventing such lobe from reaching a larger size, because in the hydatid cases, when a large amount of liver parenchyma has been destroyed in one lobe, then the remaining lobe or lobes have been able to increase very markedly in size. The reason why this has taken place would seem to be that the inhibiting effect on the total mass of liver cells present has been reduced and has remained reduced until the cells have again made up the deficiency.

It would thus appear that the proliferation or cessation of proliferation of the cells of tissues and organs is dependant in part on the mass of such tissues and organs present and on their relative relationships with the other tissues and organs of the body. Presumably the correlation between these tissues and organs is brought about by means of chemical messengers of some sort. If we can imagine a cell or a group of cells dislocated from its fellows so as to be no longer affected by these chemical messengers, then we can understand the cells proliferating indefinitely and giving rise to a tumour or a neoplasm, the cells of which are no longer under control by the other cells and tissues of the body.

In this way we see a means by which simple tumours may originate. Such simple tumours may be looked upon as originating in dislocated cells cut off in some way from their correlation with their fellows and consequently tending to show unbridled increase.

In the case of malignant tumours we may see possibly a similar process at work or possibly the explanation may be that the malignant cells had undergone a change—I would suggest a gametogenic change—by which they were giving rise to a tissue that was abnormal and was not provided for during developement and consequently for which there was no controlling mechanism available to prevent the cells multiplying unduly.

Let us return now to the two chief characteristics of malignant cells—their departure in general appearance from the normal cells of the part, though they still retain certain distinctive features of the parent cells, and their failure to be retained within their normal boundaries—in fact they show evidence of active invasive properties. From our knowledge of the behaviour of parasites, both bacterial and protozoal, it is easy to conceive that some such bodies may enter the cells, multiply in them and so alter their appearance and natural behaviour and so stimulate them without destroying them, that the above changes occur. No one, however, has clearly demonstrated the presence of such parasites nor do the facts known about the ætiology of malignant growths make it likely that their origin can be explained in this simple way. The majority of workers are united in considering that this view is essentially the correct one and with them I cordially agree, though still holding that such a parasitic origin cannot be completely ruled out of court.

It seems to me that the nature of malignant growths can be much more rationally explained by considering the ontogenetic developement of the individual. In fact, the views that I propose to lay before you to-day are such that, if the premisses on which they are founded are correct, the occasional developement of a malignant neoplasm and the behaviour and appearances manifested by the cells of such a growth, are exactly what one would expect to take place. One might almost add that had cancer never yet been met with in man, say through the premature deaths of all adults at the age of twenty, then on these views it might have been predicted that were the span of life extended by a few decades certain cells might be expected to alter their habits and to produce cell masses possessing the main features of cancerous tissues. Even now, we might prophesy that if we only lived long enough, we would all eventually suffer from such growths.

To understand these views it is necessary to revert to the very beginning of the individual's existence. We see here a pluri-potential cell, the fertilized ovum, containing within it all the potentialities afterwards manifested by its myriads of descendents. We see a cell whose progeny will form all the varied somatic cells of the body and at the same time a cell which represents the germ-plasm, that priceless jewel handed down from generation to generation and protected from the dangers and vicissitudes of the outside world in that golden casket that we know as the soma. It would seem that the whole object of the existence of the individual, in whom we recognize this soma, is to insure the safety and the handing on to the next generation of this precious germ-plasm—may we hope that it is true that in thus handing on the germ-plasm, we are able to enrich its potentialities.

The fertilized ovum after a certain number of indifferent divisions very soon shows differentiation of tissues and at an early stage we can recognize that certain of the cells are normally destined to be somatic cells and that others are the progenitors of gamete-forming cells. It has long been held by certain zoologists that there is a direct continuity of the germ-plasm from one generation to another. By this is meant that even with the earliest divisions of the fertilized ovum certain cells are set aside for the purposes of reproduction and that these cells are never ordinary body cells, even though the number of

their chromosomes be the same. I am glad to see that some biologists at least are now no longer holding to this view, though a few years ago one might have been considered a zoological heretic to have believed otherwise. In botany the issues are somewhat clearer and it has been more generally admitted that we may have an alternation of generations—a somatic individual plant culminating in the setting aside of certain somatic cells to form a gametogenic plant (with a reduced number of chromosomes) from some of whose cells the gametes are evolved. I do not think we can hold, then, the view that with the first and immediately subsequent divisions of the fertilized ovum we have a sharp differentiation between soma cells and gametogenic cells. Even those who believe that there is a direct continuity of the germ-plasm, will probably admit that not all the descendants of the cells thus pecially allocated for this purpose will form gametes and that many will be sacrificed to insure that a chosen few will be placed in the best possible circumstances for the formation of gametes and these sacrificed cells, sacrificed to protect or to nourish their chosen fellows, are essentially soma cells, at least until they have undergone a reduction of their nuclei (as in the case of the polar bodies of the maturing ovum). In vertebrates we are entitled to hold that there is just as much an alternation of generations as in plants; in fact can we reasonably think otherwise? Do not wee see in these multicellular organisms exactly what we see in certain protozoa? In an early malarial infection of the blood of man, we see a number of individually separate somatic cells, invading his red cells and multiplying somatically for a number of generations. Were these individuals associated together in colonial fashion, we would have a soma comparable with our own bodies. Ultimately, after a varying number of such somatic divisions, some of the cells, instead of continuing a somatic life, become differentiated into gametocytes and only now do we see as a crowning-point to the somatic multiplication the developement of special cells which, though individually separate, we may collectively think of as a gametogenic tissue. From the gametocytes the gametes themselves are formed. Here there is no doubt of a purely somatic phase being interpolated between gametogenic phases and of a gametogenic phase being the connecting link between two somatic phases. What of such a fundamental nature occurs in a differentiated protozoon, may be expected to occur in the metazoa, both animals and plants.

If I am right in assuming that the gametogenic tissues are derived from what are theoretically somatic cells, then we must assume that in the somatic progenitors of the sex cells we must have some factor present that, when the right time comes, will cause the cells to become sex cells and eventually will lead to the differentiation of gametes. If this be the case, then in these cells we must suppose the presence of a somatic factor which dominates the situation and moulds the cells to the requirements of the body as a whole and to the needs of the future sex-tissue in particular, until such time as the other factor present, the gametogenic one, is prepared to take charge and set about the direct formation of sex-cells. In other words, these progenitors of the gametes possess two factors, a somatic and a gametogenic one. In the early stages the somatic factor is dominant, the gametogenic held in sway and recessive, though possibly nevertheless exerting some influence over the behaviour of the cells (for example causing the invasion of the underlying tissues by columns of cells from the germinal ridge). As the cells arrange themselves in the tissues, as the ovary and testis develope, the somatic factor wanes in intensity until with the advent of puberty the gametogenic factor becomes the dominant partner and matured sex cells are differentiated.

If we associate the possession of both somatic and gametogenic factors in the antecedents of the sex cells, can we reasonably deny the existence of both these factors in all the rest of the progeny of the fertilized ovum, as long as these cells are capable of dividing? Surely not! Are we not compelled to think of all these cells as being potentially gametogenic, as each, to speak figuratively, being desirous above all things of being the transmitter of the germ-plasm to the next generation. But in the interests of this next generation it is necessary that the great majority of the descendants of the fertilized ovum should be sacrificed to make an efficient tabernacle for the protection of the chosen few destined to produce specialized gametes. We see them thus sacrificed and so ending the continuous line of living matter that traces itself directly back to the first dawn of life on this planet in the shape of the body cells. In these cells under normal conditions the somatic factor remains dominant till their end comes and governs developement, so that "fitly joined together" and working in complete harmony and coordination, the body developes and then maintains its integrity, at least until the new generation can be firmly established. But with this somatic developement and dominance, can we rightly consider that the gametogenic factor has entirely disappeared? Surely not! It cannot in the interests of the individual be expected to manifest its presence under ordinary circumstances. To do so would be of no advantage to the individual, might even be of disservice. So tenacious is the grasp on life of all living beings, so imperative is it that gametes shall be formed to establish the next generation, that we must look upon all these ordinary somatic cells as still retaining this gametogenic factor as long as they possess a nucleus and so are capable of dividing. The factor is, however recessive, held in complete subjection normally by the pronounced dominance of the somatic factor, so necessary for the harmonious developement of the soma.

Though under normal circumstances this gametogenic factor of the body cells is thus prevented from manifesting itself, there are certain conditions in which its latent capacities may be expected to assert themselves. First of all, if the extreme dominance of the somatic factor is the reason why the gametogenic one is held in check, then gradual waning of the former may finally enable the latter to gain sway. Under such circumstances when the two factors were near equilibrium, we might expect to see the cells affected manifesting both differentiated somatic features and features characteristic of gametogenic tissue or of its direct forerunner. Secondly, there is a natural tendency for all living beings, individual cells included, to prevent death (that is annihilation) by all possible means. In the case of old and effete organisms, laden with the products of decay and degeneration and scarred by many conflicts, the only hope lies in the production of a new individual by the formation of gametes.

I would suggest that the formation of cancerous tissue does represent the attempt by a group of somatic cells to become sex-forming with the ultimate production of gametes. Under the circumstances the gametes formed could not be expected to be differentiated as are the normal gametes, but would appear merely as clumsy cells with reduced nuclei incapable probably of fertilizing or even of being fertilized. The march of events in the cancerous process is exactly what we would expect if this view be correct. We see the cancerous cells invading in processes and columns the sub-adjacent tissues, just as we see the germinal epithelium burrowing into the tissues to form ultimately the specific cells of the ovary or the testis. Not only do we meet with invasive properties which are characteristic features also of the germinal epithelium, but we also find amongst the cancer cells clear evidence of reducing divisions and of the presence of heterotype and homotype mitoses. Thus the changes met with amongst cancer cells and the invasive properties of these cancer cells are very similar to the features of the cells and the invasive properties of the germinal epithelium; and if we assume that somatic cells still retain a gametogenic factor and that it is possible that circumstances may arise under which this latter factor can manifest itself, then the cancerous process is exactly the way in which we would expect such manifestation to present itself.

The Captive Tissues in Neoplasms.

In studying neoplasms and more particularly malignant growths one recognizes at once the presence of the malignant cells and also of other tissues whose cells are not malignant. These other tissues in some cases form an integral part of the neoplasm and in others they merely represent the remains of the invaded tissues. Now, when malignant cells invade a tissue it would seem that they take control of certain of the mesoblastic elements in the invaded part, whilst they destroy other tissues both mesoblastic and epithelial. The stroma of a carcinoma is very often modified from the appearance presented by the original tissues and evidently this change is the result of an impulse from the cancer cells which are, as it were, leading captive these invaded tissues and making use of them. That it is the cancer cells that cause the change is shown by the results of animal inoculation from one individual to another. A cancerous growth showing undue vascularity may, if transferred to another animal of the same species, produce again growths presenting a similar vascular appearance though the mesoblastic cells concerned belong to those of the second animal and are not, like the cancer cells, derived from the first. The scaffolding formed by the captive tissues enables the malignant cells to receive support and nourishment. It is obviously essential for the growth of the cancer cells that some such arrangement should exist.

In the case of sarcomata we find very poorly developed blood-vessels traversing the growth and linking up on the outside with the neighbouring normal vessels. In sarcomata it is considered that the vascular spaces have little more than an endothelial lining supported on the outside by the sarcoma cells. It is not suggested that the endothelial-like cells linking the vascular channels are really modified tumour cells. They are considered to be contiguous with and actually derived from the endothelial cells of the surrounding parts. Obviously we have in the sarcomata a very good example of a captive tissue in the endothelial lining of the vascular channels.

We may say, then, that more particularly in malignant growths we meet with three types of tissue, the malignant cells, the captive tissues and the invaded tissues. The captive tissues live in symbiosis with the cancer cells; on the other hand the invaded tissues are destroyed.

Super-Malignancy.

Occasionally in examining sections of cancerous growths one finds that the malignant cells or their arrangement varies in different parts of the growth. Thus in some places the cells though obviously malignant resemble more or less closely in their appearance and architecture the normal structure of the part, whilst in other portions the cancerous cells have departed much more widely from the normal appearance even to such a degree as to render it difficult to recognize the tissue from which they have been derived.

This appearance has been strikingly shown in two examples of carcinoma of the body of the uterus. In the same section may be seen cancerous processes which differ markedly in histological appearance. In places the growth appears as a typical columnar epithelioma. The cells here, though resembling the normal cells, differ somewhat in shape, size and staining reaction and arrange themselves round definite but highly irregular lumina. Adjacent to such an area may be seen carcinomatous cells of the infiltrating spheroidal type, lacking entirely any attempt at arrangement round a lumen. These appearances suggest that the latter type of malignant growth is a greater departure from the normal than the former. To this condition the term "super-malignancy" may be applied, though it must be clearly understood that this does not mean the acquirement of greater invasive properties or a graver prognosis. The term "super-carcinomatous change" might more clearly designate the condition. On the gametoid theory already outlined to explain the nature of malignant growths the appearances presented can be readily explained. In the columnar epitheliomatous type we have gametoid and somatic factors both present and both expressing themselves with the result that the latter factor is able to lead to a certain amount of differentiation and a considerable aping of the original tissue. Later, however, this somatic factor wanes still more in certain groups of cells with a consequent much more remote resemblance of this part of the new growth to the original glandular epithelium. It is possible even that a pre-cancerous state may also occur which may be designated submalignancy. In this state the somatic factor would be still by far the dominant partner and thus would lead to a close resemblance to the normal tissue, whilst the gametoid factor now aroused into some activity is responsible for departures from the normal, but has not yet gained unlimited sway or been able to cause definite infiltration. These changes in degree probably occur by definite steps or gradations, there being a sudden change in a group of cells in the downward path.

Rodent Ulcer.

Rodent ulcers are technically skin carcinomata. The most remarkable feature about them is that, though locally invasive and tending to recur if imperfectly removed, typical rodent ulcers show no tendency to involve the

draining lymphatic glands or to disseminate. It is still uncertain from what cells rodent ulcers are derived. Some consider that they arise from the basal layer of the surface epithelium, some from the epithelium of the hair follicles, some from both these sites. From a study of a number of sections I feel convinced that some cases, if not most, are derived from the epithelium of the hair follicle. Such an origin would explain readily the absence of dissemination. The cells of the hair follicle are engaged in welding epithelium cells into compact cylindrical bundles which become differentiated into the hairs themselves. We all know how perfect this welding is and how difficult it is to separate from each other the individual flattened cells that compose each hair. If a malignant growth developes from cells possessing the function of making hairs, and if some of the somatic features of such cells are still retained, then we would expect the groups of cancerous cells to adhere more firmly to each other than groups of cancer cells derived from other epithelial structures. This adherence together or partial welding would make it difficult for individual living cells or groups of cells to be detached from the masses and carried by the lymph stream or blood stream to other parts. In this way may be rationally explained the most characteristic feature of a rodent ulcer.

Dr. Reginald Webster read a paper entitled "A Study of the Incidence of the Various Serological Types of Pneumococci in Pneumonia of Childhood; Observations on Serum Therapy." The full paper has been published in The Medical Journal of Australia, February 9, 1924, page 129.

THE INCIDENCE AND MORTALITY OF PNEUMONIA AT THE ROYAL PRINCE ALFRED HOSPITAL WITH A NOTE ON ITS SERUM TREATMENT.

By Mona Ross, M.B., Ch.M., D.T.M.,
Chief Resident Pathologist, Royal Prince Alfred Hospital,
and
A. H. Tebbutt, D.S.O., B.A., M.B., D.P.H.,
Honorary Director of the Pathological Department, Royal Prince Alfred Hospital, Sydney.

Epidemiologists have in the past paid much attention to this disease, but it has been left to bacteriologists to explain many of the factors, such as transmission, virulence *et cetera* and much still remains to be elucidated.

Musser and Norris writing on this subject in Osler and McCrae's "Modern Medicine" (1907), sum up by stating that "the explanation of these facts must come from a more thorough knowledge of bacteriology. Upon such a knowledge is based the main hope for the future. The fact that improved hygienic measures have thus far failed to check the prevalence of pneumonia and that the application of antiseptic measures has been equally unavailing, indicates the limitations of our information upon the question of transmission."

With reference to seasonal influence, they quote Anders, (*American Medicine*, Volume VIII., September 3, 1904): "The major influence exerted by the seasons however is probably not direct (*e.g.* by a lowering of the bodily resistance due to low temperature, high barometric pressure, direction and velocity of the winds *etc.*), but indirect, namely, by bringing about that effective element in the causation, concentration and increased virulence of the specific poison in consequence of closed doors and windows and lack of free ventilation."

With regard to the death rate from this disease there is evidence that it has distinctly increased during the last half of the nineteenth century in such widely separated cities as Vienna, Glasgow and Philadelphia. This may be due to factors associated with increase of urban population.

Many of the questions raised as to transmission, virulence, immunity, epidemic endemic types *et cetera* have been answered and the Rockefeller Hospital workers in America and F. S. Lister in South Africa have pride of place in this field. In Australia Dr. S. W. Patterson (The Medical Journal of Australia, 1922, Vol. II., page 115) has drawn attention *inter alia* to the age-incidence and mortality of this disease at the Melbourne Hospital and mainly owing to the enthusiasm of Dr. A. Z. Huie, to whom we are greatly indebted, we are able to show similar charts drawn from the records of the Royal Prince Alfred Hospital for the decade 1910-1921. It must be borne in mind that these figures are for eleven to twelve years, whilst the Melbourne Hospital figures are for two and a half years. Our charts are built upon 1,555 diagnoses of lobar pneumonia and 691 of broncho-pneumonia.

Incidence.

Chart I. shows the distribution of all cases of lobar pneumonia into age periods (continuous line), the interrupted line showing the percentages of population in New South Wales at the same age periods in the year 1911. It will be seen that the incidence reached a maximum in the age period 20-30 (20.8% of all patients), after which it diminished almost regularly. Broncho-pneumonia had a maximum incidence in the age period 0-10 (60.4% of all patients).

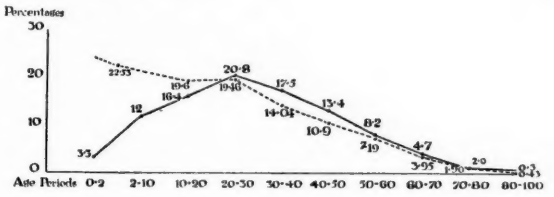

Chart I.

Distribution of cases of Lobar-Pneumonia at Royal Prince Alfred Hospital into age periods (continuous line), compared with the distribution of the population of New South Wales in 1911 into age periods (interrupted line).

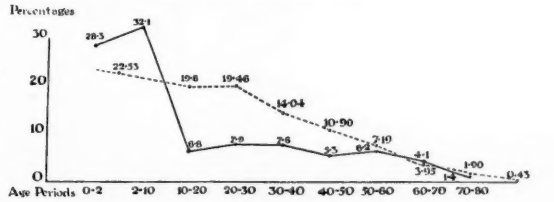

Chart II.

Same for Broncho-Pneumonia.

Mortality.

Chart III. shows that the mortality of lobar pneumonia varied considerably from year to year, namely between 10% and 21%, the average for eleven years being 17%. In the age period 10-80 the average mortality at the Royal Prince Alfred Hospital in the years 1919 to 1921 was 17% and during nearly the same period at the Melbourne Hospital the mortality was shown by Patterson to be 21.4%. The three cities Melbourne, Sydney and Brisbane are conveniently situated for a study of climatic influence on the incidence and mortality of this disease and one would expect, if the impressions of clinicians are correct, that the incidence and mortality would diminish with the latitude. The mortality from year to year of broncho-pneumonia (Chart IV.) was still more variable but was usually lower (15.5%) than that of lobar pneumonia. The variation was between 30.76% in 1915 and 7.95% in 1916.

Chart V. shows that the mortality of lobar pneumonia varied very much at different age periods (interrupted line), being high in infancy (28.3% from 0-2 years), falling to 5.26% from 2-10 years and to 1.83% from 10-20 years and thereafter rose steadily through life. The Melbourne Hospital figures (continuous line) correspond closely with those at the Royal Prince Alfred Hospital. The mortality of broncho-pneumonia (Chart VI.) was high in infancy (0-2 years 22.63%), was lowest between 2-10 years (5%) and thereafter increased somewhat irregularly throughout life.

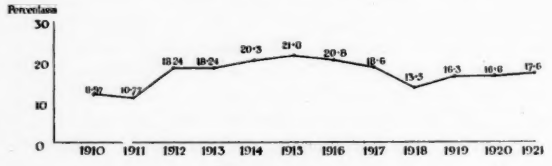

CHART III.

Annual case mortality from Lobar-Pneumonia at the Royal Prince Alfred Hospital from 1910 to 1921. Average mortality 0 to 100 years is 17.1 per cent. Average 10 to 80 years is 18.5 per cent. Lowest mortality in 1911 is 10.7 per cent. Highest in 1915 is 21.6 per cent.

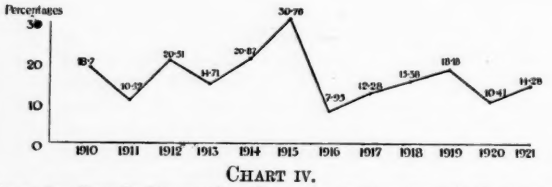

CHART IV.

Same for Broncho-Pneumonia. Note the greater irregularity from year to year. Average is 15.5 per cent. Lowest in 1916 is 7.9 per cent. Highest in 1915 is 30.7 per cent.

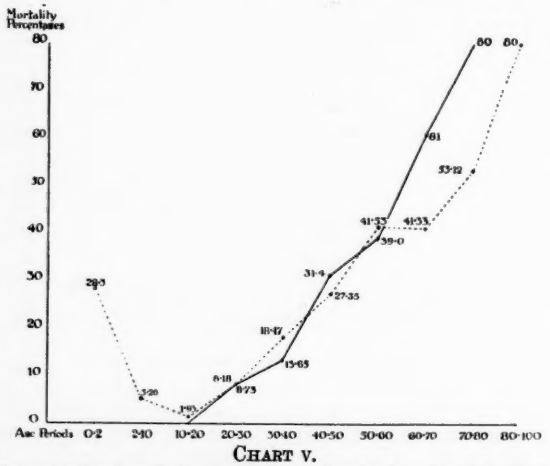

CHART V.

Case mortality from Lobar-Pneumonia at Royal Prince Alfred Hospital at different age periods (interrupted line). Note the parallelism with the Melbourne Hospital figures (continuous line) of Dr. Patterson, but mortality is higher than in Sydney (21.4 per cent. compared with 17 per cent. for approximately the same time and the same age periods (10-80 years). Note also the steady rise in mortality with years of life, excepting that the minimum is from 10 to 20 years.

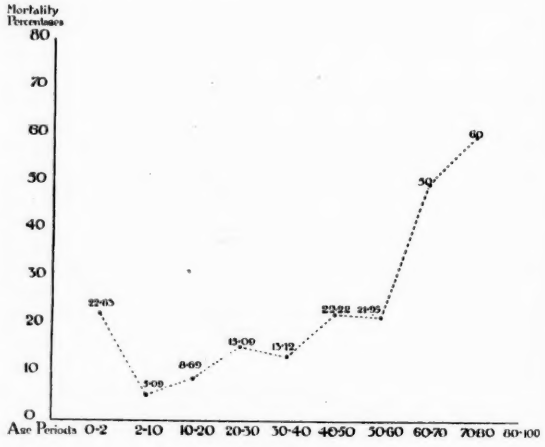

CHART VI.

Same for Broncho-Pneumonia. Note that the increase is less regular.

Empyema.

The incidence of this complication (Chart VII.) only increased slightly with age, and the mortality was not appreciably affected by it between the years ten to fifty. In early life and old age it appeared to be a more grave complication. The average incidence rate of empyema (8.7%) corresponded very closely with that found by Patterson at the Melbourne Hospital (8.6%).

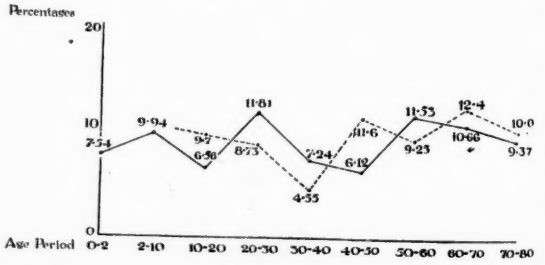

CHART VII.

Incidence of Empyema at different age periods at the Royal Prince Alfred Hospital from 1910 to 1921 (continuous line) compared with same at the Melbourne Hospital from 1919 to 1922 (interrupted line). Average for former is 8.7 and for latter 8.6, and no age period appears to be specially susceptible or immune, but speaking generally it is somewhat more common after 50 years.

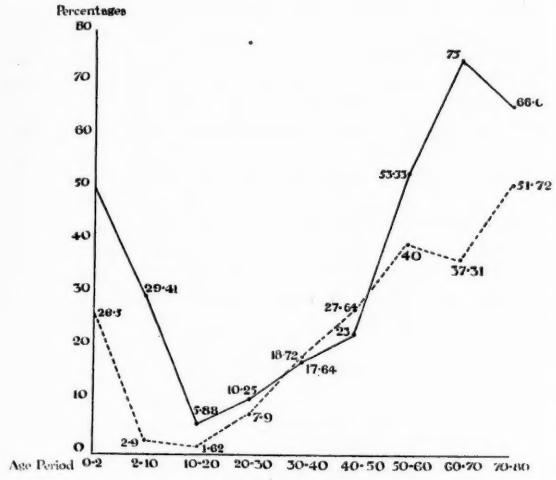

CHART VIII.

Empyema case mortality in age periods at Royal Prince Alfred Hospital (continuous line) compared with mortality in cases in which empyema was not reported (interrupted line). Note absence of important differences except in infancy and childhood and after 50 years.

Serum Treatment.

During the year from July 1, 1922, until June 30, 1923, one hundred and seventy-nine patients with acute lobar pneumonia were admitted to this hospital. Of these patients thirty-one died, a mortality of 17%, which is identical with the average for ten years.

The mortality at the different age periods is well exemplified by this year's figures (Table I.).

TABLE I.

Mortality at Different Age Periods, 1922-1923.

Result	Years							
	0-2	2-10	10-20	20-30	30-40	40-50	50-60	60-100
Recovered	1	8	26	33	33	24	16	7
Died	1	0	1	2	5	10	7	5
Total	2	8	27	35	38	34	23	12

At the beginning of July, 1922, the treatment of acute lobar pneumonia by serum was begun as follows:—

The polyvalent serum prepared at the Commonwealth Serum Laboratories was used. The cases at first were not chosen, but serum was given to each patient as soon as the clinical diagnosis was made and before any bacteriological examinations were carried out. The only preliminary step taken and one that was done in every case, was the testing of the patient for sensitivity to horse serum. This was done in the following manner. One-fifth of a cubic centimetre of a 10% dilution of normal horse serum was given intradermally and the reaction noted at the end of fifteen minutes, thirty minutes and one hour. A saline solution control was also made. When an area of erythema had developed around the inoculation together with a raised weal in the centre, serum was not given. The number found to be sensitive was much higher than was expected and serum was withheld from about one in twenty persons for this reason. To some of these patients antitoxin had been given some years previously and to two anti-streptococcal serum had been given.

If the patient were found to be insensitive, serum was given intravenously. In our earlier cases we had immediate reactions, varying in severity, taking place before the full dose of 120 cubic centimetres could be given, often rendering it necessary for us to stop the injections altogether. In our later series we found that by diluting the first thirty cubic centimetres of serum with equal quantities of normal saline solution and giving it more slowly, we could run in about ninety cubic centimetres of serum with very little immediate reaction on the part of the patient.

Blood cultures were made and sputum taken for investigation at the same time.

After the first twelve patients had been treated, the only result that could be noted was a lessening of the toxic condition of the patient, as evidenced by a fall in pulse rate and a less restless state.

A different procedure was then adopted, mainly on account of expense. The cases were selected and the serum was not given to young adults who were not showing serious toxic symptoms, or who were thought to be approaching their crises. Patients are not often admitted to our public hospitals on the first few days of the disease. In examining the records of the 179 patients admitted to the Royal Prince Alfred Hospital last year, it was found that the average duration of the disease at admission was 4.7 days. This is one condition that makes the giving of serum in hospital rather unsatisfactory. Many were admitted as late as the sixth or even seventh day, and had their crises a few hours later. Some, too, were admitted late in the disease in a moribund condition and died within twenty-four hours. After we had had some experience of giving serum to these patients with no effect in any of them, they also were removed from the list where serum was indicated.

This has, therefore, left very few patients to whom serum was given and from them it is probably unfair to draw an opinion as to the value of serum treatment in pneumonia, but the record is put before you and the treatment of pneumonia with serum will be continued until a sufficient number of cases are on record to justify an opinion as to its value or otherwise.

Table II. indicates the types of pneumococci isolated and the mortality in serum-treated cases.

TABLE II.

Result	Type I	Type III	Non-agglutinable	Mortality
Recovered	14	0	8	21.5%
Died	1	2	3	
Total Number Treated	15	2	11	

Of the fourteen patients with Type I. infection who had serum, three developed empyema. The two patients with Type III. infection died. In both cases the pneumococcus was obtained from the blood and from the

sputum. There is no evidence for or against the hypothesis that the crisis was accelerated by the administration of serum.

Table III. shows the duration of the disease and the date of administration of serum (day of admission).

TABLE III.

Patients' Initials	Day of Serum	Day of Crisis	Empyema	Day of Death
W.H.	4th	5th	—	—
C.	4th	7th	Empyema	
P.	3rd	5th	—	—
What.	6th	9th	—	—
Wr.	2nd	5th	Empyema	
Gil.	4th	Lysis	—	—
S.	6th	8th	—	—
M.	2nd	6th	—	—
Mc.	5th	10th	—	—
N.	5th	15th	—	—
T.	2nd	8th	—	—
P.	3rd	7th	Empyema	—
H.	4th	5th	—	—
L.	2nd	—	—	2nd day
H.	7th	—	—	8th day (pericarditis)
McL.	8th	—	—	8th day
Cl.	4th	—	—	5th day

The average period after onset on which serum was given, was 4.2 days.

It will be noted that the patients who died after receiving serum, died either the same day or the next day, that is to say, they were very ill or moribund and the serum failed to save them. It is highly desirable that, if serum is to be given in lobar pneumonia, it should not be left until the patient is gravely ill or moribund. The serum is not a last resort, but to be given intelligently early in the disease, the first dose being given within twenty-four hours of admission.

Therefore, the early diagnosis of pneumonia, prompt application for admission to hospital by practitioners and the immediate admission of these patients by medical superintendents are necessary, if serum treatment is to be satisfactorily adopted in hospitals. Pneumonia should be dealt with as promptly as acute abdominal conditions.

This winter (1923) nearly all the pneumonic patients admitted to the Royal Prince Alfred Hospital had a history of an initial influenzal cold and from the sputum of many patients streptococci were isolated and not pneumococci.

In examining sputum for the type of pneumococcus, it was found that mouse inoculation according to Avery's method was the most satisfactory.

The bile solubility test was used for differentiating a non-agglutinable pneumococcus from a streptococcus.

Finally we know practically nothing of the bacteriology of lobar pneumonia in Sydney prior to the last pandemic of influenza, but certainly since that time many cases have appeared to be a bacteriological problem in themselves. Because of a clinical diagnosis of lobar pneumonia, one would often be courting error to assume that it is a pneumococcal infection. The bacteriology of pneumonia cannot be taken for granted. Anatomical and clinical diagnoses such as bronchitis, broncho-pneumonia and lobar pneumonia are not final diagnoses, no more than enteritis is a final diagnosis in cases of suspected typhoid or paratyphoid fever. Routine and prompt examinations should be made of the sputum and exudates wherever found and the bacteriologist should combine direct bacteriological examinations with mouse inoculations.

REPORTS OF THREE CASES.

By W. E. J. PARADICE, M.B., CH.M. (Sydney),
Surgeon Lieutenant, Royal Australian Navy.

Inguinal Bubo Accompanied by Eosinophilia.

A certain officer reported "sick," having suddenly had a rigor, at the same time complaining of tenderness in the left inguinal region. Within a few hours he developed a definite lymphangitis, there being a hard cord-like lymphatic vessel running from a few centimetres above the internal malleolus to a superficial inguinal gland. Over the inguinal area a patch of skin about ten centimetres (four inches) in diameter became erysipeloid. "Ichthyol" was applied to this area and along the lymphatic vessel. During the next twenty-four hours further rigors occurred and the temperature was very irregular, ranging from normal to 38.9° C. (102° F.), the variations of temperature not conforming to any type and the pulse varying in accordance with temperature.

In ten days time the lymphatic condition had subsided and the patient was apparently normal.

Careful examinations failed to reveal any local lesion which could have been responsible for the lymphatic infection.

For about one month this officer remained apparently healthy and normal, but then began to suffer from general malaise.

During the next forty-eight hours his temperature rose again to 38.9° C. (102° F.) and he had several rigors. On examination two small tender superficial glands were felt in the left inguinal region. "Ichthyol" and foments were applied locally and iodide of potash was given by mouth after the first few days.

A differential blood count was made and was as follows:

Polymorpho-nuclear cells	60%
Lymphocytes (large, small and transitional)	29%
Eosinophile cells	10%
Basophile cells	1%

The noticeable feature of this is the increase of eosinophile cells and the corresponding reduction in the proportion of polymorpho-nuclear leucocytes. The eosinophile cells themselves consisted of equal numbers of small cells with very large granules and showing very little cytoplasm

and of large cells with slightly smaller granules showing a considerable amount of cytoplasm.

The above treatment was continued for eighteen days during which time the temperature continued to rise at irregular intervals and one gland apparently subsided, whilst the other continued to enlarge and become more tender.

A great number of blood counts and examinations of blood for parasites were carried out at all hours of the day and night. On all occasions differential white cell count was practically as given above and on no occasion was any parasite or any other abnormality detected.

As the response to medical treatment was unsatisfactory, it was decided to remove the glands under general anæsthesia.

At the operation the whole inguinal area was noted to be exceptionally vascular and small collections of pus were found among the loculi of fat in the vicinity of the inflamed glands. Slides and cultures were made from this pus.

A mass of fat containing three obviously pathological glands were removed.

The wound was closed and the patient made an uninterrupted recovery leaving hospital fourteen days later.

Previous History.

During the previous ten years this officer had been to various parts of the world, his last trip outside Australia being to Singapore about eighteen months previous to this illness. Whilst on this trip the patient became seriously ill and after some weeks of intermittent vomiting returned to Sydney where a laparotomy was performed. A kinked and more or less inflamed appendix was removed and adhesions surrounding the duodenum were suitably dealt with.

Course of Illness.

On leaving hospital the patient's blood contained 5% eosinophile cells and four months later 1%.

The patient's health has been average. About two months after discharge from hospital there was tenderness in the glands of the other groin, but these subsided under foments in three days.

No cause for this could be detected and the eosinophile cells in the circulation did not increase.

Examination of Material obtained at Operation.

The pus obtained from between loculi of fat contained about 50% of eosinophile cells in various stages of degeneration, the majority being apparently normal. This pus was sterile on culture.

On macroscopical examinations the glands were seen to include circular necrotic areas, the larger ones having gone on to cavity formation. The gland tissue other than the necrotic areas seemed normal and the abrupt ending of the necrotic areas was most striking.

The cultures made from the necrotic areas were sterile.

On microscopical examination the glands were found to contain collections of eosinophile cells. These collections were more numerous in the vicinity of the cavities than elsewhere.

Lymph nodes are well marked and between the nodes the gland tissue has collections of large pale cells varying in size and shape.

Some of these cells have a concentric arrangement, whilst others are collected in solid formation suggesting a new growth.

There are no hæmorrhages into gland tissue, but some degree of extra-capsular hæmorrhage is present.

The area containing the large pale cells also contained a considerable number of young blood vessels.

In this case I have not been able to determine the cause. The condition may be tropical bubo, bubo resulting from infection by intestinal parasites or filariasis.

Tropical Bubo.

There is a condition which medical officers in the service refer to by this name and in which buboes form for some unknown reason.

If these buboes are opened they take a very long time to heal, but when excised as was done in this case the condition clears up satisfactorily.

None of the medical officers who have treated such patients, can tell whether there is an eosinophilia present or not and I cannot find any mention of the condition in text books.

Bubo, the Result of Infection by Intestinal Parasites.

The last issue of *The Journal of the Royal Naval Medical Service* mentions cases of bubo caused by intestinal parasites and we know that eosinophilia is one of the accompaniments of infestation by intestinal parasites.

In this case, however, there were no other symptoms of this and I have not discovered any signs.

Filariasis.

In "Some Results of Medical Researches in the Western Pacific" by T. W. O'Connor, reprinted by the Royal Society; Tropical Medicine and Hygiene (May 18, 1922, page 37), cases are described in which the symptoms and pathological findings have a considerable resemblance to this case.

My provisional diagnosis of this case prior to operation was bubo due to filaria, my explanation of symptoms being as follows.

The first rigor with lymphangitis was due to a filaria passing into an inguinal gland. Here the parasite became quiescent and then about one month later became active for a short while and died, causing the second illness with the persistent enlargement of the glands.

Hypertrophy of Gums.

On arrival from England this rating reported to the Dental Surgeon.

He was found to have hypertrophy of the mucous membrane of the hard palate which was heaped up anteriorly into large rugæ, almost obscuring the buccal surface of the teeth. The mucous membrane external to the teeth was also hypertrophied obscuring the outer surface of the teeth.

History.

About six weeks previously this rating noticed that he had difficulty in cleaning his teeth on account of the enlargement of the gums. Since this time the gums have enlarged slightly more.

There is no pain, but recently slight tenderness to pressure has developed. This, of course, may be due to the

fact that a considerable amount of decomposing food has accumulated between the gums and teeth, but there are no signs of inflammation present.

Snippings were taken and paraffin sections cut, stained and examined.

Microscopical Appearance of Gum Specimens.

The papillae of the mucous membrane showed more layers of epithelial cells than normal, but the individual cells are normal in appearance.

The fibrous centre of each papilla is larger and denser than usual and is continuous with hypertrophied fibrous tissue between the mucous membrane and the alveolar process.

There is a small degree of small-celled infiltration at the base of a few of the largest papillae, but other than this there are no signs of inflammatory reaction.

In my opinion the condition is a simple hypertrophy of two tissues, the mucous membrane and the underlying fibrous tissue, the cells of neither tissue attempting to invade the other.

The condition is rare, neither of the Dental Surgeons at Garden Island having previously seen a case.

With regard to treatment the authors of American dental text books advised removal of the whole alveolar process. They contend that the condition starts as a hypertrophy on the alveolar periosteum.

It is worth noting that this man denies syphilis and his serum does not react to the Wassermann test.

The subsequent history of this man is most interesting.

On April 20, approximately one month after the snippings from the gum were taken, the rating was admitted to hospital with an enlarged gland at the left angle of the mandible.

On April 26 his temperature was 39.1° C. (102.4° F.) and there was a certain amount of swelling of all cervical glands.

From this time until May 9 the patient's temperature was usually 37.2° C. to 37.8° C. (99° F. to 100° F.) and various glands became enlarged.

On April 9 after being drowsy for about twenty-four hours the patient died suddenly, general lymphatic enlargements being then present.

A blood film made on April 28 showed a picture of lymphatic leuchæmia.

A *post mortem* examination was performed by Dr. Tebbutt and the following points noted. A general enlargement of lymphatic glands was present. The cervical glands were most affected. There was hyperplasia of the red marrow of the medullary cavity of the long bones. Infiltration of portion of the lungs with lymphocytes was noted, the condition resembling lympho-sarcoma.

Tht hypertrophy of the gums in this case was the first sign of an acute lymphatic leuchæmia.

Aspergillosis.

A specimen of sputum was forwarded for examination for tubercle bacilli, the only clinical notes being that the patient had a cough accompanied by considerable sputum for some time and was suspected to be suffering from tuberculosis.

Macroscopically the specimen was seen to consist of semi-transparent papyraceous plaques varying in size up to twelve millimetres in diameter and of a faint yellowish green tinge in a thin milky fluid. The plaques did not float and were of a very tenacious structure, considerable force being required to tease them out with pins.

The sputum was odourless.

Microscopically the milky fluid was found to consist of bacteria of various sorts and large numbers of Gram-positive spores, the majority of which started to grow.

The solid elements of the sputum consisted of densely matted hyphæ.

The greater part of the masses of hyphæ were Gram-negative, but small portions here and there retained the stain to a varying degree.

Scattered about in the network were masses of almost spherical spores, which did not show any signs of growing and which were strongly Gram-positive.

NOTES ON THE COMPLEMENT FIXATION TEST FOR TUBERCULOSIS AND DREYER'S ANTIGEN.

By Charles Badham, B.Sc., M.B.,
Assistant Micro-biologist, Department of Public Health, Sydney.

During an investigation into the complement fixation test for tuberculosis I have made use of living tubercle bacilli as an antigen as recommended by several authors and more recently by Punch & Gosse and later of my own preparation of Dreyer's antigen.

The sera on which this work has been done I have received by the courtesy of Dr. Palmer, of the Waterfall Sanatorium, and his assistant, Dr. Rushton Smith. This sanatorium is chiefly used for patients suffering from chronic pulmonary tuberculosis. During this investigation the following interesting facts have been noted by me and call for further inquiry. These facts are:

(1). The large percentage of sera giving a positive response to the Wassermann test carried out according to the technique of Griffith & Scott. Of eighty-four sera fifteen gave a positive Wassermann reaction. This fact may raise again the question of the specificity of the Wassermann test as done by recent cold fixation methods on the sera of tuberculous patients.

(2). That by the cold fixation method tubercle bacilli both living and defatted, owing to their containing lipoid material, are capable of acting as antigens in the Wassermann reaction. This fact has been noted by several authors in performing the Wassermann test at 37° C. and it is intensified by cold fixation methods when a highly dilute antigen gives more satisfactory fixation in the Wassermann reaction than a more concentrated one.

(3). That Dreyer's antigen is in my hands as efficient an antigen as living tubercle bacilli. So far there has not been published any account of the antigenic properties of Dreyer's antigen when tested against tuberculous human sera.

(4). That the complement fixation test for tuberculosis may be used for standardizing Dreyer's antigen.

(5). That the complement fixation test can be carried out in any laboratory where the Wassermann test is done and may be regarded as at least 70% efficient.

Methods.

Two methods were used. The first is a cold fixation method at 6°C. with an antigen of living tubercle bacilli. The second is a method of fixation at 37°C. with a suspension of Dreyer's antigen.

By the first method fifty sera were tested in eight series, the test being repeated in all cases. These sera were from patients definitely diagnosed as suffering from pulmonary tuberculosis. I endeavoured to secure sera of patients in whose sputum tubercle bacilli had been found within the past year, but in this I was only partly successful. The clinical evidence in these fifty patients was that they were suffering from pulmonary tuberculosis at different stages. Of these sera thirty-six gave a positive reaction to the complement fixation test for tuberculosis (72%) and fourteen a negative reaction (28%). Among those sera causing complement fixation with the tuberculosis antigen were nine sera which also gave a positive reaction to the Wassermann test, as performed according to the technique of Griffith & Scott.

In using any cold fixation method with tubercle bacilli as an antigen, living or defatted, the fact that these bacilli will act as a Wassermann antigen must be taken into account; this will seriously interfere with the use of the cold fixation methods in the complement fixation test for tuberculosis.

Complement.

Following Griffith & Scott's work on the varying fixability of complement being of more account than the varying haemolytic activity, I have used a fixed amount of complement in this test. With the practice of supersensitization which they advocate, the hæmolytic activity of the serum of selected male guineapigs varies very little; the amount of complement used may be taken as the equivalent of one and a half to two minimum hæmolytic doses of other workers.

Antigen.

This was prepared by washing off with saline solution a slant of tubercle bacilli grown on Dorset's egg medium (a sufficient growth is present in from three to four weeks) and shaking vigorously with glass beads. The amount of saline solution used varied from fifty and seventy-five cubic centimetres. The same strain of tubercle bacillus was used throughout and also in making Dreyer's antigen. It was isolated early this year by Miss Ethel Pinkerton of this Department from the sputum of a patient who died later from pulmonary tuberculosis. It was shown by Dr. E. W. Ferguson, Principal Micro-biologist of this Department, to be a human strain by rabbit inoculation. The antigen was used diluted so that at most it would absorb half a minimum hæmolytic dose of complement or one-third of the amount of complement used in the test, but when the protective power of a "negative" serum was considerable and the antigen was adjusted so that it was anti-complementary even to one and a half minimum hæmolytic doses or the whole amount of complement used in the test, it still gave clear cut results similar to the results obtained with a less concentrated antigen.

Technique.

The total volume used in the test was one cubic centimetre. A drop method of a type described by McDonald is relied on to deliver the reagents in equal volumes of 0.25 cubic centimetre. Pipettes were graduated by means of a Wynn and Timmin's standard wire gauge and used in a Duffy's pipette holder. Fixation was allowed to take place for eighteen hours at 6°C. On the addition of the sensitized red cells incubation at 37 °C. for half an hour followed.

By the second method with the use of Dreyer's antigen which I had prepared, I examined the sera of sixty-two patients all of whom had tubercle bacilli in their sputum. Of these forty-six gave a positive reaction to the test (74.2%), the remainder failed to react.

In this method the fixation was accomplished in the usual manner at 37°C. for half an hour. The antigen used was an emulsion of tubercle bacilli, dried and defatted as described by Dreyer until they were no longer acid fast; 0.25 cubic centimetre, of an emulsion containing 0.2 milligramme of these bacilli to the cubic centimentre was used.

This antigen was anti-complementary to the extent of less than half the complement used, which was the equivalent of two minimum hæmolytic doses. This is a difficult antigen to handle in the cold fixation method and despite the prolonged treatment with "Formalin" and acetone, it still contained sufficient lipoid substances to exhibit Wassermann antigenic properties.

By the two methods described I have tested over one hundred sera sent in for routine Wassermann test. Of these 4% gave a positive reaction to the complement fixation test for tuberculosis, that is apart from those sera which gave a positive reaction for the Wassermann test and which accordingly gave a positive reaction for the complement fixation test for tuberculosis when the cold fixation methods were used. Of this 4% I could not obtain any clinical grounds justifying the reaction.

I do not propose to review the literature dealing with the complement fixation test for tuberculosis. Several important papers have recently been published.

In conclusion I can only describe my work as a series of preliminary trials in this important investigation. After studying their articles I cannot subscribe to the statement of Punch & Gosse to the effect that their work with living tubercle bacilli as an antigen "gives merely the finishing touch" to the work that has gone before. In my hands a similar method made more sensitive by ice-box fixation has not given such good results. I think that the complement fixation test for tuberculosis will quite shortly be placed on a more sure basis. Already English authorities have reported series of tests which they consider 90% efficient, and I am optimistic that with further work I could get much better results than those I have so far obtained.

The Preparation of Dreyer's Antigen.

In the preparation of this antigen which I commenced on the receipt of *The Journal of Experimental Pathology* in August last, I used tubercle bacilli which had been isolated from a human being. The rate of growth was slow. The cultures used in August were two months old. They were made by Mr. R. Grant of the Department of Public Health. The process of treating the bacillary mass with "Formalin" for four hours at 100°C. and acetone for twenty-four hours at 65°C. in a Soxhlet apparatus, had

to be repeated three and four times before the great bulk of the bacilli were no longer acid fast. Unfortunately Dreyer gives no rule for testing this acid-fast character. I was finally content with defatted bacilli in which only a very small percentage retained their colour after fifteen minutes' treatment in 20% sulphuric acid. It may be that laboratory strains of tubercle bacilli frequently sub-cultured will prove much easier to treat and deprive of their acid-fast quality. As I have mentioned before the strain of bacilli which I used, was only isolated early in the year and had been sub-cultured but two or three times before it was seeded on glycerine veal broth.

COMPLEMENT FIXATION TEST IN TUBERCULOSIS

By Lucy M. Bryce, M.B., B.S., B.Sc.,
and
Miss F. E. Williams.
From the Walter and Eliza Hall Institute of Research.

This test has been carried out in the Walter and Eliza Hall Institute for about twelve months. One of us (Miss Williams) has done the actual serological work and the other (Dr. Bryce) has endeavored to correlate the results obtained with the clinical findings in order to gain some idea of the value of the test up to date.

Technique.

With the exception of the antigen the reagents and methods are identical with those used in the Harrison method of performing the Wassermann test.

Summarized briefly, these are as follows.

Haemolytic Systems.

The hæmolytic system is a 6% suspension in normal saline solution of washed sheep's cells with equal parts of titrated and suitably diluted hæmolysin obtained from serum of rabbits previously injected with sheep's cells.

Complement.

Serum is used from healthy adult male guinea pigs, titrated against sensitized red cells in order to find out its minimum hæmolytic dose.

Test Serum.

The serum to be tested is obtained in usual way. Five to ten cubic centimetres of blood are required. It is placed in the incubator for twenty minutes; the clot is then separated and the serum in contact with the clot is placed on ice for twenty-four hours. The clear serum is then pipetted off, centrifugalized, if necessary diluted one in five, heated to destroy complement and stored on ice till required. It is inactivated again on the day of test.

Antigen.

Many different antigens have been tried by various workers. A. L. Punch, in an article on this subject in *The British Medical Journal* (September 22, 1923) quotes Miller's classification of these antigens into four groups which are as follows:

Group 1.—Suspension of whole or ground up bacilli.

Group 2.—Antigens which may be classified as tuberculins.

Group 3.—Extracts of derivatives of tubercle bacilli.

Group 4.—Extracts of normal or tuberculous tissues.

From his survey of the literature and his own experience Punch has found that antigens belonging to Group 1 have proved the most satisfactory. The antigen which we have been using here, also belongs to this group. Others have been tried on the same sera at the same time, but have proved less satisfactory than our antigen of group 1 type, which is made from a 1% suspension of dead tubercle bacilli in carbolized saline solution.

The method is as follows. Strains of bacilli of human type obtained from various sources are grown on glycerine broth till the cultures are of suitable size. The broth is then poured off and the bacilli killed in the flask by heating in an Arnold steam sterilizer at 100° C. for one hour. The mass is then removed from the flask by the addition of a little saline solution and thoroughly dried by pressure filtration and subsequently by being placed in a slightly warm oven. This material is then ground up and emulsified in carbolized (0.5%) normal saline solution in the proportion of one part bacillary mass to ninety-nine of saline solution. This is allowed to stand and is subsequently centrifugalized till the supernatant fluid is quite clear. This is done because the presence of bacilli in the fluid is found to have strong anti-complementary action.

The features of this antigen which have proved satisfactory, are that it does not give pseudo-positive results with syphilitic sera and that a majority of the positive reactions have been strong (P + + +) *id est* more than six minimum hæmolytic doses.

In view of the recent publication of Dreyer's work, we should perhaps mention that on one occasion an antigen was made by his method, but as it was thought at the time that the cultures available might not be suitable, this was done rather to gain experience of Dreyer's technique than to assess the value of his antigen. It did not prove in this instance nearly as good as the carbol-saline extract, frequently failing to give a reaction when the latter gave a "P + + +" reaction.

In the main test four rows of tubes are set up, row 1, 2 and 3 each containing the same amounts of serum and antigen (0.1 cubic centimetre, that is two drops from a 0.05 cubic centimetre dropping pipette), but the amount of complement is varied, rows 1, 2 and 3 containing three, four and a half and six minimum hæmolytic doses respectively, the complement having been previously diluted so that three minimum hæmolytic doses are contained in one volume. The total volume in each tube is equalized by addition of the necessary amount of saline solution. The fourth row contains three minimum hæmolytic doses of complement and no antigen, being the serum control. Antigen controls containing 0, 0.1 and 0.2 cubic centimetres of antigen in first, second and third rows respectively are also put up. Pooled positive tuberculous and syphilitic sera are always included in the series tested and a Wassermann test is done on every serum tested for tuberculosis. This method is illustrated in Table 1.

By Harrison's method racks set up as above are incubated for one hour at 37° c.; sensitized red cells are then added and a further incubation is given for one hour or until all controls have completely hæmolized.

TABLE I.—Arrangement on Reagents in Final Test.

	Test				Controls		
Row	Saline Solution Drops	Serum	Antigen	Complement Drops	Saline Solution	Antigen	Complement Drops
1	2	2	2	2	6	0	2
2	1	2	2	3	4	2	2
3	0	2	2	4	2	2	3
4	4	2	0	2	4	2	4

The fourth row is the serum control.
After incubation for 1 hour, two drops of sensitized red cells added.
After further incubation for one hour, readings are taken.

In the ice box method which has also been tried on some of the sera and to which I will refer again, the racks are placed in the ice box over night, red cells are added next morning and a further incubation for one hour or till controls have hæmolysed is given.

In reporting the results to clinicians any reaction short of complete inhibition of hæmolysis in row one is given as "negative" and in compiling the following figures I have considered such readings as "negative" also.

Correlation with Clinical Data.

The number of individual sera which have been tested is 622. In some cases, however, more than one examination has been made of serum from the same patient and in others no details of the histories have been available, so that in the analysis given below only 503 cases have been considered.

The sera tested include: (i.) Serum from persons proved to have tuberculosis, the proof accepted being the demonstrated presence of the tubercle bacillus in the sputum, urine, cerebro-spinal fluid. These sera have been obtained from patients at the Melbourne Hospital, Children's Hospital, Greenvale Sanatorium and Austin Hospital. Dr. de Crespigny was also good enough to send us sera from some very interesting cases at the Adelaide Hospital. (ii.) Serum from patients with clinical or suspected tuberculosis, when the clinician sending it has wanted confirmation of his opinion. By its value in such cases as these the test will ultimately stand or fall, as clinically it is obviously unnecessary after bacilli have been found in the sputum or elsewhere. (iii.) Serum sent for other tests, such as the Wassermann, hydatid or gonococcal complement fixation tests. These have been tested with tuberculosis antigen to establish the specificity of the latter.

I have, therefore, classified the sera, when the clinical data have been sufficient to do so, into four groups:

(1) Proved tuberculosis.
(2) Clinical tuberculosis.
(3) Suspected tuberculosis.
(4) Non-tuberculous conditions.

This is not an entirely clear cut and satisfactory classification, as there are some cases in group (2) which I would like to be able to place in group 1. Group (2) and group (3) also obviously overlap, but I have, as far as possible, sought the cooperation of the clinicians under whose care the patients were. Dr. F. B. Lawton especially has been very helpful, in sending a large number of serum specimens from suspected tuberculosis from his Out Patient Clinic.

(1) *Proved Tuberculosis.*

The test has been carried out with the serum of seventy patients, in fifty-three of whom a reaction was obtained, 75.7%.

An interesting point is that in twenty-nine of these fifty-three, that is in more than 50%, the reaction was "P + + +."

The figures are given in Table II.

Table II.—Proved Tuberculosis.

"P + + +" (fixing six or more minimum hæmolytic doses of complement) ..	29	
"P + +" (fixing four and a half to six minimum hæmolytic doses)	9	
"P +" (fixing three to four and a half minimum hæmolytic doses)	10	
Positive with Wassermann reaction	5	
Total Positive	53	= 75.7%
No reaction	17	
	70	

These fifty-three patients all had pulmonary tuberculosis, but in one the bacillus was found in the cerebro-spinal fluid, the patient being admitted with meningitis; the *post mortem* examination revealed old apical caseation and cavitation and a recent miliary tuberculosis involving the lungs and meninges.

The seventeen patients whose serum did not fix complement have been analysed, but I have not been able to find a common factor to account for their failure to react. They include individuals of various ages, whose illness varied in duration from two months to several years and whose local signs varied from those of slight apical involvement to those of well marked, actively progressing disease. In some there were signs of general toxæmia (slight pyrexia, malaise, night sweats and so on) and some were in a very advanced stage in which the cause of failure was probably the asthenic condition of the patient. Patients who from this last cause failed to react to the von Pirquet test, have not in our series given a positive complement fixation. These seventeen

patients all had pulmonary lesions, some complicated by involvement of other parts of the body, such as cervical glands and bones. A larger series would possibly leave a smaller percentage of unexplainable failures, as this group includes several examples of cases found by other workers to yield "negative" results, that is early and very advanced disease.

(2) *Clinical Tuberculosis.*

In this group are cases regarded clinically as certainly tuberculous, but in which the presence of the tubercle bacillus has not been demonstrated and which, therefore, for the sake of strict accuracy cannot be classified as proved tuberculosis. It includes three sub-groups. In the first are patients with pulmonary disease in whom for various reasons no bacilli have been found in the sputum. The total number of these is forty-eight of whom twenty-two, (*id est* 45.8%) gave a positive reaction.

The figures are shown in Table III.

cases of pulmonary tuberculosis, but also a number of other, suspected of being tuberculous only at the time when the serum was sent and later, proved to be non-tuberculous. As the group is large and the number which have been definitely proved not to be tuberculous is, as yet, small, I have not attempted to tabulate the group in greater detail. Examples of cases which proved non-tuberculous is cited.

A patient was admitted to Melbourne Hospital with a pleural effusion. In the course of investigation his blood was sent to us for the complement fixation test and a "P +" was returned by the Harrison method, although by the ice box method we failed to confirm this reaction. The patient subsequently died and *post mortem* examination revealed a mediastinal malignant growth. This is the only case in which one of those already proved to be non-tuberculous has given a positive reaction. Another case of suspected tuberculosis was that of a boy who, after

TABLE III.—Clinical Tuberculosis.

Reaction to test for tuberculosis	Pulmonary	Hip	Spine	Other Bone	Miliary (including Meningitis)	Glandular	Renal	Peritoneal
P++	5	1	1	—	—	—	1	—
P++	2	—	—	—	1	—	—	—
P+	14	1	1	—	—	1	—	—
Positive and Positive Wassermann reaction	1	—	—	—	—	—	—	—
Total Positive	22	2	2	—	1	1	1	—
Negative	25	9	8	2	2	2	—	1
Negative and positive Wassermann reaction	1	—	—	—	—	—	—	—
Total Negative reaction	26	9	8	2	2	2	—	1
Total number of cases tested	48	11	10	2	3	3	1	1

In the patients who failed to react, there was a similar wide range of difference in duration, general condition and so on, as in the proved group.

All patients with clinically certain tuberculosis of bone are included in this group and in them a low percentage of positive results has been obtained. This is possibly related to the fact that no bovine strain has been used in the preparation of the antigen.

The third division is a heterogeneous group, too small to be of any statistical value. It includes three patients with miliary tuberculosis of whom one yielded a "P + +" reaction, one patient with tuberculosis of the kidney who yielded a "P + + +" reaction, three patients with glandular tuberculosis, one associated with pulmonary tuberculosis, one with tuberculosis of the tarsus and one with uncomplicated tuberculosis. The first of the three yielded a "P +" reaction, while neither of the other two reacted. Finally there was one patient with chronic adhesive tuberculous peritonitis, diagnosed at operation. No reaction was obtained.

Summarizing the above, we find that of forty-eight patients with pulmonary tuberculosis twenty-two or 45.8% yielded a reaction, of twenty-five with bone tuberculosis four or 16% yielded a reaction and of eight with various forms of tuberculosis three or 37.5% yielded a reaction. This gives a total of eighty-one patients of whom twenty-nine or 35.8% yielded a reaction.

(3) *Suspected Tuberculosis.*

This is a large group, including probably most early typhoid fever had most persistent intermittent pyrexia for some weeks. Generalized tuberculosis was suspected and his blood sent for complement fixation test; no reaction was obtained. He subsequently recovered with no evidence of tuberculosis. Many of these patients present such indefinite clinical syndromes, including perhaps only one or two of the symptoms of early tuberculosis, that no definite conclusion can be drawn from them. There is among this group a number from the Out-Patients' Department of the Melbourne Hospital whom it will be interesting to keep under observation for the next year or more, to ascertain whether they subsequently develope proved tuberculosis and if they do, at what stage in their illness the complement fixation test first yields a reaction (see Table IV.).

(4) *Non-tuberculous Conditions.*

This group includes those sera sent for the hydatid or Wassermann test, on which the tuberculosis complement fixation test has been applied for the purpose of controlling it and establishing its specificity.

Of these none gave a greater reaction than "P +" and only three gave this reading. In one there was no evidence of tuberculosis, a second was insufficiently investigated, while the third was suffering from pernicious anæmia and *post mortem* there was at the apex of the right lung a small old caseating tuberculous focus. One hundred and seventy-five failed to react to both the tuberculosis and Wassermann tests, while forty-four gave a positive Wassermann reaction.

Specificity.

With Regard to Wassermann Reaction.

A positive Wassermann reaction together with complement fixation with tubercle bacilli antigen has been present in five patients proved to be suffering from tuberculosis in two of whom a history of syphilis was also present.

A reaction was also obtained in a patient with typical apical tuberculosis. The presence of bacilli was not recorded and the positive Wassermann reaction was probably incidental. It was also obtained with the serum of a patient with pleurisy with effusion in whom tuberculosis was strongly suspected, and in whom there was also evidence of syphilis. Of seven patients, therefore, whose sera have reacted to both tests, there is evidence that both diseases were present in three and the possibility of crossing in four. But against this ninety-four sera fixing from three to six or more minimum hæmolytic doses with the tubercle antigen have fixed none with the Wassermann antigen, while fifty-six sera giving a positive Wassermann reaction have not given any reaction with tubercle antigen.

With Regard to Hydatid Disease.

The three complement fixation tests for hydatid disease, tuberculosis and syphilis have been done with 158 sera. Of these eighteen which yielded a complement fixation reaction for tuberculosis, also yielded a reaction for hydatid disease but none to the Wassermann test. The three sera which yielded a reaction both in the Wassermann test and in the hydatid complement fixation test, and belonged to persons known to have both diseases, failed to react to the complement fixation test with tubercle bacilli antigen.

There can be no doubt about the specificity of the complement fixation reaction for tuberculosis when this technique is used.

TABLE IV.—Suspected Tuberculosis.

Reaction to test for tuberculosis	Pulmonary	Pleural	Renal	Miliary	Peritoneal	Glandular	Spine	Hip	Other bones	Skin	Unknown
P+++	3	1	—	—	—	—	—	—	1	—	
P++	1	—	1	—	—	—	—	—	—	—	—
P+	4	—	—	—	—	—	1	—	—	—	1
Positive and positive Wassermann reaction	1	1	—	—	—	—	—	—	—	—	—
Total positive	9	2	1	—	—	—	1	—	1	—	1
Negative	70	14	3	5	1	4	2	1	2	1	9
Negative and positive Wassermann reaction	12	—	—	—	—	—	—	—	—	—	—
Total with negative reaction	82	14	3	5	1	4	2	1	2	1	9
Total number of cases tested	91	16	4	5	1	4	3	1	3	1	10

Comparison Between Ice Box and Harrison Methods.

The above results were those given by the Harrison method only which has been used for all the sera tested. The ice box method has been used in conjunction with the Harrison in approximately three hundred cases. From the results obtained, it appears that the ice box method is slightly more sensitive, giving some positive results in proved, clinical and suspected tuberculous sera with which the Harrison method failed, but on the other hand, it appears to give pseudo-positive results with syphilitic sera and with sera, otherwise not reacting from patients in whom tuberculosis was not suspected. Table V. gives the results in tabular form.

TABLE VI.—Pleural Fluids.

No.	Chest Fluid		Serum		Affection
	Harrison Method	Ice Box Method	Harrison Method	Ice Box Method	
153	P+++	–	P+±	–	Inflammatory effusion, Wassermann reaction (+++), husband tuberculous.
156	Neg.	Neg.	Neg.	Neg.	Repeated effusion, probably malignant.
157	P+±	–	Neg.	Neg.	? Tuberculosis, Wassermann reaction (+++).
256	Neg.	P+	Neg.	Neg.	? Tuberculosis, fever, severe sweats, vague broncho-pneumonic signs.
416	Neg.	Neg.	Neg.	Neg.	? Tuberculosis.
442	Neg.	Neg.	Neg.	Neg.	? Tuberculosis.
578	P+	P+ tr.	Neg.	–	? Tuberculosis.
M	P++	–	P++	–	? Tuberculosis.
522a	P+±	P+±	Not examined		? Tuberculosis.

TABLE V.—Comparisons between Harrison and Ice Box Methods.

Both "negative"	273
Harrison method positive (P or over), ice box method "negative"	1
Harrison method "negative," ice box method positive	14
Proved tuberculosis	2
Clinical tuberculosis	2
Suspected tuberculosis	5
Unsuspected	2
With Wassermann reaction	3
Both positive to same degree	47
Both positive (Harrison method stronger than ice box method)	2
Both positive (ice box method stronger than Harrison method)	21

Body Fluids.

Body fluids other than sera have been examined in some cases, chiefly pleural fluids, and occasionally cerebro-spinal and synovial fluids. In these cases we like to test a sample of serum at the same time. The fluid has been diluted 1 in 5 and inactivated on day of testing.,

The results obtained with the pleural fluids and sera are shown in Table VI., while the results with pleural effusions in which the sera only were tested are shown in Table VII.

TABLE VII.—SERA FROM PATIENTS WITH PLEURAL EFFUSION.

No.	Methods		Affection
	Harrison	Ice Box	
9	Neg.	Neg.	Recurrent effusion—probably malignant disease.
111	Neg.	Neg.	Small pleuritic effusion after appendicectomy.
175	P+±	—	Pneumonia, with delayed resolution and continued slight pyrexia; also cardiac failure.
18	Neg.	Neg.	? Tuberculosis.
176	P+	Neg.	Recurrent effusion, mediastinal malignant disease.
507	Neg.	Neg.	? Tuberculosis; inoculated guinea pig has enlarged inguinal glands.
351	Neg.	Neg.	Pleural effusion in case of nephritis.
516	P±	P+±	Pleural effusion, tubercle bacilli in sputum.
519	Neg.	Neg.	? Tuberculosis; Wassermann reaction (+++).
522	Neg.	Neg.	? Tuberculosis; Wassermann reaction (++ tr.).
529	Neg.	Neg.	? Tuberculosis.

DR. W. CAMAC WILKINSON referred to some work that he had performed on complement fixation in tuberculosis and quoted the recent important work of Engel and Bauer on this aspect of diagnosis. These and other workers attributed the effects of the action of the tubercle bacillus to the disintegration of the endotoxins and urged that some of the complement fixation phenomena might be produced by varying immunity products. The speaker emphasized the fact that immunological methods of diagnosis often gave misleading results because the immunity reaction to tuberculosis was not necessarily humoral but cellular. He was much more impressed by the use of tuberculin as a diagnostic method.

DR. F. GUY GRIFFITHS said in reference to some remarks by one or two clinicians that instead of criticising the pathologists for the present incompleteness of their results, not yet claimed to be final, the clinician should be grateful to them for their scientific work which was so interesting and so likely to be very helpful in the future. The speaker referred to the first application of Bordet and Gengou's complement fixation reaction by Wassermann and Bruck to tuberculosis and how their attention had been diverted to syphilis. The work on tuberculosis was kept alive, however, by Arloing and Courmont and others and the speaker hoped that the present wave of enthusiasm would lead to great and important results.

DR. GORDON HISLOP said that he spoke as a clinician and that in his opinion a positive complement fixation test could not be taken as an indication of activity of the tuberculous lesion nor could it by itself be taken as a proof of infection by the tubercle bacillus, but that in conjunction with other tests, clinical, radiological and bacteriological, it could be regarded as a distinct help in the diagnosis of early tuberculosis.

DR. A. H. TEBBUTT stated that he wished to emphasize the fact that Wassermann reaction was unlike the tuberculosis complement fixation test in that it was an index of infection and not of immunity. The Wassermann test was reliable clinically, but many of the other fixation tests were not nearly as accurate from the clinical point of view. He referred to the uncertain and indefinite results in gonococcal infection confined to the anterior portion of the urethra. In complicated cases, such as gonococcal arthritis, the test was much more reliable. He referred to the work which had been performed at the Walter and Eliza Hall Institute on the Casoni reaction and suggested that in the future some such correlation of the cutaneous reactions in tuberculosis with the fixation test might be discovered. He asked the clinician to bear this in mind and by further investigations to help to throw light on the various problems of immunity in tuberculosis.

DR. W. J. PENFOLD stated that he was interested to know whether there was any definite correlation between the complement fixation and the von Pirquet reactions. He also wished to know whether any experimental work on the immunity reactions in animals had been performed.

DR. C. BADHAM congratulated Dr. Bryce on her paper and referred to the lack of understanding on the part of the clinician. He was convinced that if the study of these reactions was persevered with, great advances would be made.

DR. BRYCE replied shortly. With regard to the questions put by Dr. Penfold she stated that as far as could at present be stated there was no obvious correlation between the von Pirquet and the fixation reactions. No experimental study of the reactions in animals had as yet been performed at The Walter and Eliza Hall Institute.